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A STUDY OF RABIES, WITH REFERENCE TO A NEURAL TRANSMISSION OF THE VIRUS IN RABBITS, AND THE STRUCTURE AND SIGNIFICANCE OF NEGRI BODIES *

ERNEST W. GOODPASTURE, M.D.

DEPARTMENT OF PATHOLOGY, VANDERBILT UNIVERSITY, NASHVILLE, TENNESSEE

(From the Institute for General and Experimental Pathology of the University of Vienna, Acting Director: Professor Dr. T. C. Rothberger)

PART I

It has been previously shown that when a virulent neurotropic strain of the virus of herpes simplex is inoculated in sufficient quantity into the right masseter muscle of rabbits, one can demonstrate the first resulting cerebral lesion within the corresponding fifth motor nucleus. The virus affects especially the ganglion cells without demonstrable lesions in the right fifth motor nerve throughout a wide extent of its extracerebral course.[1] For this and other reasons it is believed the herpetic virus enters the brain from a peripheral focus of infection through the axis-cylinders of nerves supplying the infected area. There is also strong anatomic and experimental evidence for believing that the virus of rabies enters the brain through the nerves which supply the area wounded by the bite of a rabid animal, though the prevailing view seems to be that the virus gains entrance to the central nervous system through perineural lymph spaces [2, 3, 4, 5].

Several points of similarity between the infections of the central nervous system caused by the herpetic and the rabic viruses suggested that one might be able in like manner to demonstrate the first lesions of rabies in the ganglion cells of the fifth motor nucleus

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on the same side, if one inoculated the virus into a masseter muscle. In this way like evidence might be gained that in rabies as in herpes the virus is conducted to the brain along the axis-cylinders of nerve cells.

Both herpes and rabies induce within cells of the central nervous system structural changes which render these infections especially adaptable to cytologic investigation. Recent studies have shown that in early herpetic lesions characteristic intranuclear bodies appear, the presence of which often renders it easy to follow the course of the infection.[6, 7] In the case of rabies, the presence of typical Negri bodies in cells of the nervous system has been regarded for several years as pathognomonic of the disease. Thus we have in the one case intranuclear and in the other intracytoplasmic bodies stainable by suitable methods; and it was hoped that the latter might serve like the former as guide-posts along the infected paths. For example, if one succeeded in demonstrating Negri bodies within the ganglion cells of the right motor nucleus following an inoculation of rabic virus into the right masseter muscle, and in no other cells of the nervous system and if lesions were not found along the course of the right fifth motor nerve, it might reasonably be supposed that the virus had entered these cells through axis-cylinders rather than through perineural spaces. Because the motor nuclei of the fifth cranial nerves lie deep in the pons, it can hardly be imagined that virus would affect these cells first if it were borne only by a perineural fluid.

Experiments were accordingly undertaken with this object in view. Rabbits were chosen as the experimental animal because they are very susceptible to infection with rabies; they are easy to handle; and from previous experience it was known to be feasible with their small brains to make a study of the entire fifth motor nuclei in serial sections. These considerations overbalanced the possible disadvantage that, as is well known, Negri bodies are relatively small in rabbits infected with rabies.

It was necessary to use street virus for the study in view of the established fact that in infections with fixed virus the Negri bodies are always very small and in a high percentage of cases have not been demonstrated at all. In the beginning it was a question whether one would be able regularly to infect rabbits with rabies by an injection into a single masseter muscle, as this site for intramuscular

inoculation has not apparently been tried before. The situation of choice has been into the muscles of the back on both sides of the vertebral spines, large quantities of infectious material being injected to insure a successful result. The high percentage of successful inoculations reported following the latter procedure, however, encouraged the hope that a single injection of a considerable quantity of material into the masseter muscle on one side, especially as it is relatively near the brain, would be sufficient to infect the animal. This proved to be true.

A most important consideration was to find a suitable method for staining the Negri bodies; one that could be easily applied to a number of sections at a time in as much as it would be necessary to cut the fifth nuclei serially; and one that would readily exhibit the smallest and earliest forms. The methyl blue eosin method of Mann,[8] which was employed by Negri, was tried, but it was found to be necessary to follow the original instructions of Mann and stain the sections twenty-four hours in order to demonstrate small forms. In addition to the long time necessary for staining, each section required careful manipulation. The results with tissues fixed in Zenker's fluid by injecting the fluid through the carotid arteries were disappointing in that the sections took too deep an eosin stain, requiring long decolorization in alkali alcohol. They rarely accepted sufficient blue to afford a pleasing contrast so that the bodies could be easily detected.

The eosin-methylene blue method of Lentz[9] was not so satisfactory for our purpose as was that of Mann. Besides requiring much manipulation of slides, it did not give a sharp staining of the Negri bodies with tissues fixed in Zenker's and Helly's fluids. The method is recommended especially as a means of demonstrating clearly the "Innenkörper" and since the presence of these structures has been relatively uncommon in the bodies as found in the rabbit's brain, the method was not satisfactory for the purpose in view.

The eosin-methylene blue method of Mallory[10] was employed and gave good results with Zenker-fixed tissue in staining the larger bodies, but the smaller ones either were not stained or were obscured in early stages by the deeply staining Nissl substance.

After some experimentation with various dyes, the following method was devised which has, on the whole, given much more satisfactory results than any of the others. The only deviation from

the usual procedure in preparing sections for staining was the use of a 1 per cent solution of corrosive sublimate in 95 per cent alcohol to remove the iodin after solution of the mercury precipitate in tissues fixed in Zenker's and Helly's fluids. This technic has been followed for some years and seems with the following method to heighten the selectiveness of the stain. The carbol anilin fuchsin solution has already been described by the writer.[11] The method in full is as follows:

1. Fix fresh tissues in Zenker's fluid for 24 hours. The method is not applicable to tissues fixed in Helly's fluid.
2. Stain sections cut from paraffin 10 minutes in the following solution:

Alcohol 20%	100.0 c.c.
Phenol (pure)	1.0 c.c.
Anilin oil	1.0 c.c.
Basic fuchsin	0.5 gm.

   The finely powdered or granular dye dissolves easily and the solution is immediately ready for use. Exposure to light and air causes a precipitation of the dye in about two months so that the solution becomes weaker and must be discarded. It is convenient to keep the required alcoholic solution of fuchsin, and to add the phenol and anilin oil when it is desired to make up the stain anew. If sections are stained in the solution for an hour or more they gradually become brown and useless. In our experience it is safe to stain the sections as long as one half hour but the best results are obtained within ten minutes.
3. Wash off the excess stain in running water, blot with filter paper and decolorize in 95 per cent alcohol until the sections become pink.
4. Wash off in water and counterstain 15 to 60 seconds with Loeffler's methylene blue.
5. Wash in water. Dehydrate and decolorize for a few seconds in absolute alcohol (until the excess blue is removed).
6. Clear in xylol; mount in balsam.

By this method the Negri bodies are stained a sharp, bright red against a pale blue background. "Innenkörper" are blue, nucleoli, fibrin and red blood corpuscles are red and Nissl substance blue. One can readily detect the smallest forms of Negri bodies. In the brain of a rabbit infected with rabies, minute forms are far in excess of the larger ones, especially in early stages. "Innenkörper" are not always stained by this method, but vacuoles are evident.

A strain of street virus was obtained from a dog's brain preserved in 50 per cent glycerol for four days, and probably had begun to autolyse before being placed in glycerol. About 1 gm. of this material was washed in salt solution, ground in a mortar and suspended in 10 c.c. of saline solution. Then 2 c.c. of the suspension

were injected into the right masseter muscle of each of two normal adult rabbits. Both of these rabbits developed rabies and from the brain of one, the strain of virus used in subsequent experiments was derived. The clinical history of the disease in this animal is recorded as follows:

R 1. Normal adult rabbit.

Nov. 18, 1924. Received 2 c.c. of saline suspension of dog's brain infected with rabies (street virus). The material was injected through a large needle into the right masseter muscle. When the needle was well into the muscle the injection was made a little at a time into several different places and the muscular tissue repeatedly punctured by moving the needle point about.

Nov. 20. Rectal temperature 41 C. Right cheek not swollen. Slight lacrimation of the right eye.

Nov. 22. Temperature 41.2 C. Purulent conjunctivitis of both eyes. Cornea clear. Cheek not swollen.

Nov. 24. Temperature 41.2 C. Conjunctivitis, both eyes.

Nov. 26. Temperature 40.0 C. Conjunctivitis, both eyes.

Nov. 28. Temperature 39.5 C. Conjunctivitis improved.

Nov. 30. Temperature 39.2 C. Conjunctivitis improved.

Dec. 4. Temperature 39.2 C. Emaciated, salivated, weak.

Dec. 6. Temperature 39.8 C. Very thin and weak.

It hops with great difficulty, humping its back in a peculiar manner and falling over in the attempt. On falling, the hind legs seem especially weak, remaining extended, and the animal is unable to get up. No evidence of paralysis. No infection in the nose; eyes healed; no diarrhea. Killed with chloroform. No gross pathologic changes were found in the organs at necropsy. The brain was removed aseptically and about 1 gm. from the region of the right Ammon's horn was ground in a mortar, suspended in 6 c.c. of saline solution and injected into the right masseter muscle of each of two adult rabbits, R 2 and R 3. The medulla, pons and a portion of the Ammon's horn were fixed in Helly's fluid. The remainder of the brain was preserved in 50 per cent glycerol.

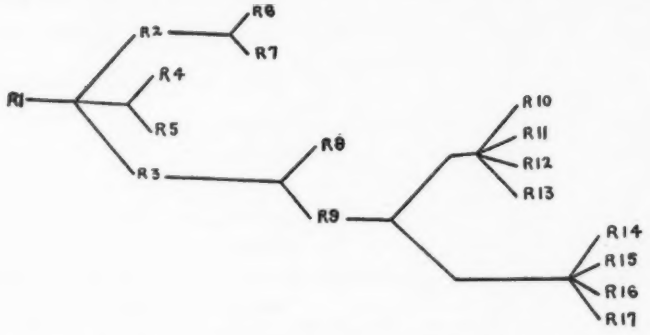
Microscopic sections from Ammon's horn and the pons show numerous Negri bodies of various sizes, often fairly large, in ganglion cells and a perivascular infiltration especially prominent in Ammon's horn.

In this animal there was evidently a primary bacterial infection associated with purulent conjunctivitis which caused the early elevation of temperature. It is interesting that no fever accompanied the symptoms of rabies. This was also true of the second rabbit receiving dog's brain. In the two animals (R 2 and R 3) inoculated with fresh brain of R 1 there was a slight elevation of temperature following the inoculation, which soon became normal and was fol-

lowed by a second rise with the onset of symptoms of rabies. In rabbits inoculated from these, there was no initial elevation of temperature but fever always accompanied the onset of symptoms usually appearing before other symptoms were apparent.

In the accompanying chart the animals used in these experiments are numbered and the source of the virus in each instance is recorded. All the animals were inoculated with a suspension of brain injected into the right masseter muscle.

All of these rabbits except one (R 7) developed rabies. This rabbit inoculated with glycerinated brain from R 2 showed no



symptoms for twenty-three days and was killed without an examination of the brain. R 6 inoculated at the same time died of rabies on the twenty-second day.

Only nine of the remaining sixteen animals were permitted to live long enough for the development of obvious symptoms other than an elevation of temperature. Of these, five showed paralysis of the front legs affecting the right leg three times, the left leg once and both legs once. There were no evidences of paralysis in other muscles. Of the remaining four animals, two were inoculated with dog's brain and ran a slow course without the development of any paralytic symptoms; one was inoculated with glycerinated brain from R 2 and ran a slow course without the development of paralysis; and one (R 9) showed a complete loss of muscular control without evidences of localized paralysis.

Thus in this small group of experiments the paralytic symptoms indicate definitely a more pronounced effect of the virus in the cervical portion of the spinal cord following an inoculation into the

right masseter muscle. This is particularly true on the side in which the injection is made but the result is not constant. In no instance, however, has a localized paralysis been observed in the hind legs.

Microscopic sections present a less definite finding and the lesions are difficult to interpret. It is clear that the injury is most marked in the pons, medulla and cervical cord, but the relative injury on the two sides seems in most instances about the same with possibly a greater severity on the right side.

Several rabbits were killed at various stages of the disease in order to study the first lesions, especially as they were manifested in the fifth motor nuclei. It was found that material from the third rabbit passage caused no preliminary elevation of temperature but fever was the first evidence of infection. Two series of animals were therefore studied, R 10 to R 17, after inoculation with the brain of R 9, to determine the earliest lesions. R 13 was killed on the fifth day after injection and before there was an elevation of temperature. R 12 was killed on the seventh day within twenty-four hours after the first elevation of temperature; R 17 on the twelfth day within twenty-four hours and R 11 on the eleventh day forty-eight hours after the first elevation of body temperature. R 12 and R 17 showed no other evidence of disease than fever. R 11 showed beginning paralysis in the right front leg. The protocol of R 9 given in Part II will illustrate the latest stages.

In addition to these animals, the protocols of which will be used to illustrate the others, serial sections were cut and the fifth motor nuclei studied in R 3, 4, 5, 8 and 9. The results conform to those described in the following protocols, although in R 3, 5 and 9 late stages were present.

R 13. Normal adult rabbit.

Jan. 5, 1925. Received 2 c.c. of a suspension of fresh brain from R 9 into the right masseter muscle.
Jan. 8. Temperature 39.0 C.
Jan. 9. Temperature 39.3 C.
Jan. 10. Temperature 39.3 C. Condition good, appears normal. Killed.

Brain injected through the carotid arteries with Zenker's solution. Serial sections through the fifth motor nuclei were stained by the eosin-methylene blue method of Mallory and the carbol anilin fuchsin method. The pons appears normal. The Nissl substance in the ganglion cells of the fifth motor nuclei is of normal appearance. No Negri bodies or other evidences of injury or infection are found.

R 12. Normal adult rabbit.

Jan. 5, 1925. Received 2 c.c. of a suspension of fresh brain from R 9 into the right masseter muscle.
Jan. 8. Temperature 39.2 C.
Jan. 9. Temperature 39.3 C.
Jan. 10. Temperature 39.3 C. Condition good.
Jan. 12. Temperature 40.4 C. No lameness, paralysis or other evidence of disease. Killed.

Brain injected with Zenker's fluid. Serial sections through the fifth motor nuclei were stained with eosin-methylene blue (Mallory). The cells appear in every way normal. No Negri bodies found.

R 17. Normal adult rabbit.

Jan. 27, 1925. Received into the right masseter muscle 2 c.c. of brain suspension from R 9 (twenty-two days in glycerol).
Jan. 30. Temperature 39.5 C.
Feb. 3. Temperature 39.3 C.
Feb. 4. Temperature 39.6 C.
Feb. 5. Temperature 39.5 C.
Feb. 6. Temperature 39.4 C.
Feb. 7. Temperature 39.4 C.
Feb. 8. Temperature 39.2 C. Appears normal.
Feb. 9. Temperature 40.3 C. Appears normal. Killed.

Brain injected with Zenker's fluid. Microscopic sections were made through the frontal lobes, midcerebrum including Ammon's horns, the brain stem, cerebellum, medulla and cervical cord. In addition, serial sections were made through the fifth motor nuclei in the pons. Sections were stained by the carbol anilin fuchsin method.

Very minute Negri bodies, or "lyssa bodies" according to the distinction made in the second part of this paper, are found sparsely distributed in ganglion cells of each part of the brain and cord, excepting the cerebellum. They are not demonstrable in the cells of Ammon's horns. Nissl substance is well stained generally.

The "lyssa bodies" are most numerous in many ganglion cells of the fifth motor nuclei. They are very small, homogeneous spheres and appear to involve more cells on the right side than on the left, but the difference is only relative.

Although the bodies are more numerous and in more cells in the fifth nuclei, there is already an early perivascular infiltration in the neighborhood of the tenth nuclei in the medulla and about ventral blood vessels in the cervical cord. The inflammatory exudate is not found elsewhere.

This case demonstrates the rapid spread of the virus through the central nervous system once it has entered, and convinces one of the futility of using the Negri bodies with this strain of virus as an in-

dex of the earliest site of infection in the brain and cord. It seems evident that by the time the bodies become demonstrable, the virus has already spread perhaps throughout the nervous system.

R 11. Normal adult rabbit.
Jan. 5, 1925. Received into the right masseter muscle 2 c.c. of an emulsion of fresh brain from R 9.
Jan. 8. Temperature 39.3 C.
Jan. 9. Temperature 38.9 C.
Jan. 10. Temperature 39.5 C.
Jan. 12. Temperature 39.6 C.
Jan. 13. Temperature 39.2 C.
Jan. 14. Temperature 39.2 C.
Jan. 15. Temperature 40.1 C. Appears normal.
Jan. 16. Temperature 40.2 C. Eats. Right ear hangs down. Head inclines slightly to the right. Easily excitable. Cannot support itself on the right foot which is definitely lame. Killed.
Brain injected with Zenker's solution. Serial sections through the fifth motor nuclei were stained by the carbol anilin fuchsin method.

There is a severe inflammatory reaction characterized by perivascular and tissue infiltration throughout this series of sections involving to about the same degree on each side of the pons, only the motor and accessory motor nuclei. Both sensory roots of the fifth cranial nerves are also inflamed to the point where Schwann's sheaths appear, more conspicuously on the right side where perivascular and focal cellular infiltrations are noted especially prominent just central to the sheaths of Schwann.

Negri bodies are very numerous in the ganglion cells of both motor and accessory motor nuclei, but are not found elsewhere in these sections. Most of them are quite small, appearing as spheres or ovals and often containing vacuoles. No Negri bodies are found in the cerebellum just above the pons.

Discussion

An examination of the above protocols shows that it has not been possible to prove by the methods used that Negri bodies appear first in the ganglion cells of the right fifth motor nucleus following an inoculation of the virus of rabies into the masseter muscle on the same side. It is evident, however, that an inoculation of fairly large quantities of the virus into a masseter muscle causes an infection of the brain in a high proportion of cases. With the strain of virus used, infection took place within a relatively short time, shorter

with fresh rabbit's brain than with dog's brain or glycerinated rabbit's brain.

From an anatomic standpoint there is no conclusive evidence that the virus enters the brain through nerves. The clinical course of the disease on the whole, however, may be best explained on the theory of a neural transmission of the virus from the site of inoculation, an idea founded upon much careful anatomic and experimental work. In five cases out of nine permitted to live long enough for paralytic symptoms to appear, paralysis in the fore legs was evident in three on the right side, in one on the left and in one on both sides. No local paralytic involvement of the hind legs was observed in any of these experiments. In one animal of the nine, no localized paralysis but a general loss of muscular coördination was observed. In three there was late general muscular weakness but no complete loss of control. One of the nine rabbits showed no evidence of rabies.

The paralytic symptoms, therefore, show that following an inoculation into the right masseter muscle, the anterior extremities are first affected and more often on the right side.

Histologic evidence based upon the presence of exudate, Negri bodies and degenerative changes in ganglion cells indicates that the virus, when it once enters the central nervous system, spreads with great rapidity. So far as the pons is concerned, the lesions may be relatively more pronounced on the right side and in the motor nucleus, although both sides were always involved when demonstrable changes were found.

In R 17, killed within twenty-four hours after the first elevation of body temperature, all the "lyssa bodies" found were of the smallest form. Their distribution indicates that even in this early period of the disease the virus had spread possibly throughout the central nervous system. This animal presented no other symptom than fever. In this case, one also observed the greatest evidence of inflammation to be situated in the medulla and cervical cord, although the "lyssa bodies" were apparently more numerous in the ganglion cells of the fifth motor nuclei. It has been usual in these experiments to find the severest lesions in the pons, medulla and cervical cord. This experience is in opposition to the view that the base of the cerebrum, that is Ammon's horns, is the site of predilection for the virus of rabies as indicated by Negri bodies. A similar view was also held regarding the localization of herpes virus, but it has been

conclusively shown that if the virus of herpes simplex enters the central nervous system through one of the peripheral nerves, the lesions at the base of the brain are always secondary and represent an extension of the virus from a lesion at the entrance of the peripheral nerve involved. This appears probably to be the case also with this strain of lyssa virus. It is true that in the first rabbit passage lesions seemed most marked in Ammon's horns. In this rabbit the progress of the disease was relatively slow and the symptoms mild. The virus had not, one might suppose, become well adapted to the new host and time was given for well marked lesions to appear at this point of secondary distribution. However, when more virulent rabbit's brain was used for the injections, the lesions were always more marked in the pons, medulla and cervical cord, which is the segment presumably nearest the point of entrance of the virus.

It is to be noted that in every case in which sections were made through the fifth motor nuclei, "lyssa bodies" were present in the ganglion cells, and if the virus entered the brain only through the right fifth motor nerve its course toward the spinal cord was much more injurious and destructive than its progress toward the cerebrum. The relatively more intense inflammation of the medulla and cervical cord suggests that the cells of these parts are more susceptible to injury by the virus, or that the virus may enter this region through sympathetic nerves. Unfortunately the cervical ganglia in these cases were not studied, but the sympathetic innervation of the muscle inoculated affords a possible portal of entry into the cervical cord if the virus is capable of progressing along axis-cylinders.

In favor of the passage of the virus along axis-cylinders, it seems to us, is the fact that in all except the very earliest cases there is evidence of injury in the central portion of the sensory division of the fifth cranial nerves. In earlier cases the cells are more or less extensively infiltrated and especially just internal to the plane where Schwann's sheaths appear. This lesion is always bi-lateral but more pronounced on the right side. In the latest stages disintegration of axis-cylinders is seen in this segment of the nerve. In one instance a loss of substance and a focal cellular infiltration occur. Coincidently there appears a very destructive lesion in the corresponding Gasserian ganglion proportional in extent to the in-

volvement of the sensory root. This lesion was studied in five cases including early and late stages. In all stages there is necrosis of ganglion cells and in the latest stage almost complete destruction of these cells, although in the early cases no evidence of inflammation along the extracranial course of the nerve is found.

In the most advanced case, various degenerative changes in axis-cylinders and a perivascular infiltration are seen in the nerve in the neighborhood of the ganglion. Evidences of injury always appear first within the ganglion cells, not in the surrounding tissue. These cells may undergo complete necrosis without cellular or other exudate about them. The inflammatory reaction which later occurs is focused about individual injured cells. These ganglion cells are surrounded by a mantle of cells whose cytoplasm and fibrils enclose them and seem to protect them much in the same way that the myelin sheaths isolate the axis-cylinders. Yet it is not the surrounding cells that show the first evidences of injury. It is known that the virus is present in such ganglia. Negri bodies are demonstrable in many of the cells, as Negri himself first demonstrated after inoculating the virus into the brain. It seems much more probable that the virus proceeds from the brain along the axis-cylinders of the sensory division which offers such a broad expanse in the pons and medulla for receiving an infection than, as suggested by Koch, that in a hypothetical stage of generalized infection the virus localizes in the Gasserian ganglia as it does in the brain. The lesion in these ganglia is in our experiments much more extensive than has been observed in other cranial or spinal ganglia, indeed they are the only ganglia in which necrosis of nerve cells is found.

These experiments illustrate the fact that Negri bodies or "lyssa bodies" may occur in great numbers within ganglion cells entirely in the absence of evidences of inflammation in the form of cellular or other exudation. It seems quite evident that cellular exudation is dependent upon the degree of injury produced by the virus and is not a necessary accompaniment of its presence. The presence of Negri bodies is an earlier manifestation of the activity of the virus than cellular exudate, yet these bodies in turn appear only after the virus has spread far to other parts. Indeed Negri bodies may persist for a relatively long time within ganglion cells without causing necrosis. Thus the action of the virus upon the cells of the fifth motor nuclei is relatively slow and mild as compared with its effect

upon the nerve cells of the Gasserian ganglia. The bodies may be present in the fifth motor nuclei at the time of the first appearance of fever, yet after three or four days of fever and shortly before the animal is about to die of the disease, it is rare to find actual necrosis, though degenerative changes may be marked. In the Gasserian ganglia, however, most of the cells die without the appearance of Negri bodies. This is in striking contrast to the action of the virus of herpes simplex. This virus rapidly causes necrosis of the ganglion cells which it first attacks, so that it is possible to find, for instance, almost complete necrosis of the cells in the right motor nucleus following an inoculation of the virus into the right masseter muscle before demonstrable lesions appear in the left nucleus or elsewhere.

Thus it appears that the presence of Negri bodies is the result of the action of the virus upon the cell and is not an essential feature of the disease. One may see cells both in the brain and in the Gasserian ganglia which show all degrees of disintegration and not infrequently necrosis, yet in the absence of Negri bodies, as has been frequently observed by other writers. In fact, in the Gasserian ganglia where the virus is most destructive, these bodies are relatively scarce in comparison with portions of the brain where necrosis is rarely observed. The cells of the brain, for example the ganglion cells of the fifth motor nuclei, evidently offer a much stronger resistance to the action of the virus than those of the Gasserian ganglia and perhaps than those of the ventral horns of the cervical cord. This very resistance may be responsible for the development of Negri bodies in greater numbers. The impression has been gained that the various structural changes observed in ganglion cells including Negri bodies are the result of the action of the virus upon the cell and the reaction of the cell to the resulting injury. It is believed that neither the virus itself nor a characteristic structural change which may be regarded as a constant accompaniment of the virus has yet been demonstrated.

The material which was prepared for this study has been utilized in a cytologic investigation of the nature of the cellular changes with especial reference to the structure and significance of the Negri bodies. The results are recorded in the following paper.

PART II

Before Negri described the bodies, which now bear his name, in the cells of the nervous system of animals infected with rabies, several investigators had noted various degenerative cellular changes associated with the lesions. These were regarded as general in their nature, that is to say, non-specific for the disease. After Negri's [12] very valuable discovery many observers believed with him that one had now in an easily demonstrable form at least a phase in the developmental cycle of the true parasite of rabies, protozoan in character. Subsequent studies, however, have gradually undermined Negri's hypothesis until nowadays perhaps no one believes that these structures, whose important practical significance is everywhere recognized, are really protozoa. However, many students of rabies, perhaps most, have not disassociated the Negri bodies from what they believe to be a visible form of the real virus. That is, they consider them structures with which the virus is in some way actually united, giving to them a specific morphology. Thus Volpino,[13] Babes [14] and adherents to the clamydozoan hypothesis hold that the minute basophilic structures within the Negri bodies constitute the specific portion and represent the true virus, while the eosin-staining portion is a sort of capsule probably produced by the cell as a hyaline mantle by which the virus may be in a measure isolated. These "Innenkörper" and the vacuoles in which they lie were carefully studied by Negri and considered by him to be a part of the internal structure of his protozoön.

Another view, perhaps less strongly supported though no less clearly defined, is well expressed by d'Amato [15] and by Lentz.[16] They maintain that the Negri bodies are purely degenerative in nature, that is, they are the product of the action of the virus upon the cellular substance but bear no essential structural relation to the virus itself.

When this study was undertaken we had no preconceived view regarding the essential nature of Negri bodies other than that their presence was considered pathognomonic of the infection. After a short study, however, of the eosin-stainable structures in the ganglion cells of rabbits infected with rabies, it became a serious question what could safely be regarded as a Negri body and what not; for it was very evident that the majority of these cytoplasmic

bodies conforming in other respects to those described by Negri were quite hyaline and apparently homogeneous and presented no "Innenkörper" stainable by the methods ordinarily used for their demonstration.

Negri himself warned that if the bodies were too deeply stained with eosin by the Mann method they often appeared homogeneous, but with a little experience in differentiating the sections an inner structure could be demonstrated. Yet he seems to have had his difficulties in this respect for in his illustrations which were reproduced presumably from characteristic preparations, one sees apparently homogeneous bodies without central vacuolation along with others which exhibit the typical structure. These homogeneous forms are especially numerous in his preparations from the brain of rabbits. Lentz emphasizes the difficulty of demonstrating "Innenkörper" by the Mann method and devised an eosin-methylene blue stain to detect them with greater precision. Schiffmann [17] recognized three types of Negri bodies, the members of one group including the smaller forms which were homogeneous and contained no demonstrable inner structure. Finally d'Amato, using a most refined technic for the demonstration of "Innenkörper," has described in the dog Negri bodies which were quite uniformly hyaline. Babes recognized similar forms.

In our preparations from the rabbit's brain one may find in the same section typical bodies with vacuoles and central "Innenkörper" and others of equal size, sometimes in the same cell, which are apparently homogeneous. It was thus necessary to admit that in rabies there occur, in addition to the bodies with "Innenkörper," other forms in all other respects similar, yet not having this differentiation. But it did not seem justifiable to group these undifferentiated forms with true Negri bodies, as others have done, in view of Negri's original description and the practical as well as theoretical importance of the distinction.

One must judge from Negri's papers that he himself regarded the presence of "Innenkörper," or the vacuoles in which they lie, as essential constituents of the type which he considered the protozoön of rabies. However, he admitted, perhaps because of the filterability of the virus (Remlinger),[18] the probability of smaller forms which could not be distinguished, owing to their size, from secretion granules or products of cellular disintegration. But for the larger struc-

tures on which his studies were based the presence of "Innenkörper" or corresponding vacuoles was essential. This is further demonstrated by the fact that in studying the Gasserian ganglia of rabbits infected with rabies, he encountered the difficulty of finding, in addition to the characteristic types, bodies which stained with eosin by Mann's method but which were irregular in size and shape containing apparently no "Innenkörper." Consequently, he was of the opinion that these more or less irregular eosin-staining structures were degenerative products of the cell, perhaps from Golgi's apparatus. These forms have also been observed by us and will be referred to later.

An exact definition of Negri bodies is an extremely important practical point in as much as their presence is used routinely to diagnose rabies in suspected animals. Fortunately the practical difficulty is not so great as it might seem, because in the brain of dogs infected with rabies larger forms of the bodies with conspicuous "Innenkörper" are usually in evidence. The necessity for demonstrating in each case the vacuolated character of suspected bodies or the presence of "Innenkörper" within them, is illustrated by the descriptions by Standfuss [19] and by Lentz [16] of structures in the brain of dogs with *Staupe*, similar to Negri bodies, excepting that they are homogeneous and occur extracellularly as well as intracellularly.

Thus for practical reasons and from the descriptions given by Negri himself one is forced to conclude that an accurate definition of Negri bodies must include the presence of "Innenkörper." According to such a definition it was evident that the great majority of the eosin-staining bodies demonstrable in the nervous system of rabbits infected with our strain of virus were not Negri bodies. For present convenience they have been termed "lyssa bodies." They have all the characteristics of Negri bodies excepting a demonstrable inner structure. It is believed, as will develop later, that "lyssa bodies" are in all essential respects similar to Negri bodies except for a different morphologic configuration.

The "lyssa bodies" are to be distinguished from the eosin-staining granules described by d'Amato in ganglion cells in cases of rabies and other conditions. These amorphous granules have been observed in this series of rabbits. They are of approximately uniform size and are arranged in the cytoplasm in rather dense groups often

at the pole of a cell or near the nucleus. These granules seem to be as d'Amato suggests, an early stage in the formation of pigment. The "lyssa bodies" are also to be distinguished from *Staupe* bodies by their intracellular situation and association with Negri bodies and the virus of rabies. They vary greatly in size, but in our experience both the smallest and the largest cytoplasmic bodies found in rabbits belong to this group. The smallest are spherical, while the larger ones are often irregular in shape with smooth surfaces sometimes penetrating into the cellular substance as if a molten material had been poured into the cytoplasm. They stain in every respect identically with the eosinophilic substance of Negri bodies by the methods of Mann, Lentz and the eosin-methylene blue method of Mallory, but they have been most easily and sharply stained, as have also the Negri bodies, by the carbol anilin fuchsin method described in the first part of this paper. This method has been used especially in the study of their origin and significance.

The distinction between Negri bodies and "lyssa bodies" has been emphasized because it has been possible to demonstrate a degenerative change in certain ganglion cells affecting the neurofibrillar apparatus in the tissues at our disposal previously fixed in Zenker's solution and stained by the carbol anilin fuchsin method. This degenerative change results in the formation of bodies identical in size, shape and staining property with "lyssa bodies." It has also been possible to observe the initiation of structural transformations in the mitochondrial substance of the cells and their processes which make it seem very probable that both Negri bodies and "lyssa bodies" have a common origin in degenerative changes in the mitochondrial and neurofibrillar apparatus of the cell, and are in no essential respect representative of the actual virus.

The changes in the neurofibrillar apparatus are found in the pons especially affecting the large ganglion cells of Deiter's nuclei. The animal in which they occurred ran a stormy course and was permitted to live until almost moribund so that the most extreme lesions might be studied. The protocol is as follows:

R 9. Normal adult rabbit.
Dec. 24, 1924. Received into the right masseter muscle 2 c.c. of an emulsion of fresh brain from R 2.
Dec. 29. Temperature 38.8 C.
Dec. 30. Temperature 39.0 C.
Dec. 31. Temperature 39.7 C. Appears in every respect normal.

Jan. 2, 1925. Temperature 40.3 C. Weak and somewhat unsteady but not lame.

Jan. 3. Temperature 40.3 C. Very unsteady.

Jan. 5. Temperature 38.7 C. Extremely weak; complete loss of muscular coördination; cannot stand, falls to the right; struggles. Fresh corneal ulcers; some salivation. Killed with chloroform and exsanguinated. Brain removed aseptically and placed immediately in Zenker's solution.

Microscopic sections, serially through the pons including the fifth and eighth nuclei, were stained by the carbol anilin fuchsin method.

There is a moderate general perivascular infiltration most marked about the large vein through the accessory fifth motor nucleus on each side. In the ganglion cells of the fifth motor nuclei are numerous Negri bodies and "lyssa bodies" usually quite small. These bodies are found in ganglion cells quite generally throughout the section and to an equal extent on each side. The Nissl substance as a rule is fairly well preserved and stains sharply. There is a very marked destruction of axis-cylinders and a cellular exudate in each sensory root of the fifth nerves. Negri bodies are numerous in the cerebellum above the pons.

The most significant changes in this case are found in the sections through Deiter's nuclei. Most of the large ganglion cells which receive fibers from the vestibular ganglia show considerable granulation and a pale staining of their Nissl substance. However, only an occasional "lyssa body" is to be found except in certain cells. The remarkable feature is that in many cells in each Deiter's nucleus there is evidence of a very acute necrosis, with what appears to be a coagulation of material in the cell body and all its processes. There appears as a result a network of fibrils staining deeply red and having the form and arrangement of neurofibrils. Various stages of this curious cellular change may be observed. In the first stage one can see deeply red-staining, often anastomosing fibrils occupying the center of a large dendritic process surrounded by a thin zone of granular basophilic material resembling the granular cytoplasm within the cell. As one follows the dendrite centrifugally the axial fibrillar cord soon resolves itself into a thin, discrete, deeply staining thread, eventually so thin, as the distance from the cell increases, that it becomes barely visible with highest magnification. Following this change into the cell one observes the group of anastomosing fibrils occupying the dendritic process spread in fan-shape into many smaller fibrils penetrating the cytoplasm. These

fibrils are largest and apparently most numerous about the periphery; in the interior about the nucleus they are very fine and may appear interrupted. The change is a uniform one affecting all dendritic processes alike. The network of fibrils within the cell seems to have contracted similarly to fibrin in a clot. The network is drawn away from the periphery of the cell leaving a distinct zone containing no fibrils and being partly empty and partly filled with basophilic granular cytoplasmic material of which strands and clumps still adhere to the original cellular margin. The Nissl substance is no longer recognizable as such, but perhaps it is represented by the basophilic finely granular, ground-substance in which the fibrils lie. The nucleus is often indistinctly outlined. Usually one can see an amorphous mass of blue-stained material (*Kernkappe?*) situated at one side and upon the nuclear membrane. The volume of the nucleus is diminished; the nucleolus is well preserved but tends to stain basophilically; and basophilic granules appear within the nucleus (Fig. 1).

In what one may regard as a somewhat more advanced stage, the fibrils in the dendritic processes are still clearly seen, but within the cell body they are broken into fragments and very small, often spherical structures (Fig. 2). A few fibrils about the periphery are more intact. It seems evident that in such a cell there was a preliminary contraction of the network, since the peripheral margin of fibrils is drawn away from the outer cellular border with granular basophilic material and clear spaces between. In some cells the earliest visible fibrils are extremely small and threadlike, in others they are much coarser as if several fine fibrils had cohered.

In the latest stages observed, the entire network of fibrillar material has become broken up throughout the whole extent of the cellular processes as well as in the cell body into round or oval, sometimes elongated, masses always with rounded contour (Fig. 3). These bodies vary greatly in size. Within the bundle of axis-cylinders entering the pons from the vestibular ganglion, which is preserved in the sections, one can observe with the greatest clearness the various stages in this interesting change as it affects the axis-cylinder (Fig. 4). Most of the axis-cylinders are entirely unstained. In those that are altered, it is first to be observed that they retain a pink coloration, and in the same axis-cylinder a succession of changes may be followed through a short segment to complete breaking up

into red-stained balls. The normally unstained portion shades into pink, then into a deep red disclosing several apparently anastomosing fibrils. The red fibrils seem to melt together forming a single deeply stained fiber, sometimes incorporating clear spaces corresponding to the meshwork of the preceding stage. There is finally complete coherence, then irregularities appear and the fiber breaks up, or rather seems pulled apart, forming spheres and oblong rounded masses. At this final stage one can discern a delicate unstained membrane which seems to be the remnant of a sheath of the axis-cylinder within which, as in a delicate tube, the balls and masses are arranged at close intervals along its course. Occasionally the axis-cylinder breaks up into innumerable minute bodies, though this is not common.

Throughout the cross-section of the pons at this level one can find numerous cellular processes of various sizes from large dendrites or axis-cylinders to delicate thin threads representing terminal processes which present the above described change. The different stages of it may be followed to complete breaking up into spheres or granules corresponding in size to the process from which they came. The granules and balls are often numerous and may appear to lie free within the gray substance extracellularly, but one can always satisfy oneself that they all arise through the destruction of a ganglionic process.

The change is most conspicuous in the bundle of axis-cylinders from the vestibular ganglion on each side. Many of these axis-cylinders are affected and stain sharply, contrasting in a striking manner with the neighboring unstained processes. Only the central terminal portions of these axis-cylinders are affected, and the change does not extend quite to the periphery. The extrapontine portion of the nerve appears superficially normal.

There are great numbers of "lyssa bodies" of various sizes in cells throughout this level of the pons. Their staining properties so far as has been observed, are identical with those of the altered fibrils and axis-cylinders as well as the bodies to which they give origin. With Mann's stain, the eosin-methylene blue method of Mallory, Giemsa's solution and with acid fuchsin-methyl green, the two substances stain precisely alike. No vacuoles or "Innenkörper" have been demonstrated in the bodies that result from the disintegration of the neurofibrillar apparatus.

With the carbol anilin fuchsin method the normal neurofibrils do not stain, but in the presence of the above mentioned degenerative change fibrils having the arrangement and general appearance of the neurofibrillar structure, as revealed by the Bielschowsky method, become intensely stained by the fuchsin. They do not appear precisely like normal neurofibrils in that in dendritic processes and sometimes about the cellular periphery they are thicker and give the impression that they are melting together and anastomosing. The nature of this change is not clear, and no description of such a transformation of the neurofibrillar material has been found in the literature. Significant and apparently related alterations of the fibrillar substance associated with rabies have, however, been described.

Bielschowsky [20] states that neurofibrils are very easily affected by a variety of injurious influences and he has classified under three headings the pathologic changes in this substance, which may occur under various degenerative and inflammatory conditions of the central nervous system. These groups are an increase in volume of the fibrils, fragmentation or granular disintegration and a hyalinization of the cellular body in which fibrillar material apparently becomes converted into a homogeneous substance. In none of these degenerative changes is there a similarity to the above described alteration associated with rabies, in which the fibrillar material of both the cell body and its processes becomes converted into structures so like the Negri bodies. Bielschowsky describes these changes as affecting principally and usually only the fibrils within the cell, and emphasizes that those of dendrites and axones resist injurious influences much longer. Nor do descriptions of degenerative changes in the primitive fibrils of axis-cylinders following section of a nerve, which were studied most carefully by Mönckeberg and Bethe,[21] offer an analogous series of events. In as much, however, as these studies were made with a different staining method it was thought best for the purpose of comparison to restudy the degeneration of axis-cylinders in rabbits in tissues fixed in Zenker's solution and stained by the carbol anilin fuchsin method. The sciatic nerves of four rabbits were accordingly cut and the peripheral and central ends were studied after two, four, six and nine days. The changes correspond to those described by Mönckeberg and Bethe and by others, that is, a granular disintegration and fragmentation, and in no way reproduce the appearances found in rabies.

Thus, so far as the literature of the subject has been reviewed, the degeneration of the neurofibrillar substance of the cell above described has not previously been noted. Its especial significance for the pathology of rabies rests in the fact that this material under the influence of rabic virus may be transformed into bodies individually identical with "lyssa bodies" and with the eosinophilic substance of Negri bodies. The cells, however, in which this change has been observed appear to be necrotic and it is a transformation which affects the entire neurofibrillar material of the cell, so that in this very essential respect the bodies which are formed differ collectively from those that lie within the cytoplasm of apparently living cells.

This complete transformation, however, is not the only change that affects the neurofibrils in the ganglion cells of rabbits with rabies. There is evidence that only a part of the fibrillar substance within the cell body may be so changed and converted into "lyssa bodies" by a similar transition. This evidence is based, it is true, on the ever-treacherous transition forms, but it is believed significant nevertheless. Bielschowsky describes a partial granular degeneration of neurofibrils affecting definitely circumscribed areas within the cytoplasm under other pathologic conditions.

Using the silver method Ramon y Cajal [22] and Babes have studied the neurofibrillar material in rabies and have described very distinct changes usually associated with the severer degrees of injury. They observed an increase in volume of fibrils corresponding to Bielschowsky's first type of pathologic alteration. This increase in volume is associated with a diminution in number of fibrils, so that it is difficult to say whether it is due to an actual enlargement of individual fibers or to a melting together of several originally separate ones (Bielschowsky). Babes found such fibrils in the anterior horn cells of rabid dogs, ten or more microns thick. He observed that they became altered in their staining property in that they failed to become impregnated with silver and were hardly recognizable as fibrils except that one could trace them into the normally staining fibrils of the cellular processes. He further noted that they sometimes became apparently completely homogeneous or dissolved in the cytoplasm. This stage probably corresponds to Bielschowsky's third type of pathologic alteration. The altered staining property of the material offers a difficulty with the silver method in following

any possible transition of this substance into "lyssa bodies" since they, as well as the eosin-stainable portion of Negri bodies, do not retain the silver. Using the carbol anilin fuchsin method on the other hand, one gets the positive reproduction of the picture, that is, the swollen neurofibrils begin to take the fuchsin stain. It is thus possible to observe transitions which suggest very strongly that many of the larger "lyssa bodies" are formed by a fusion and a breaking up into a coarse network or irregular masses of the neurofibrillar material. This type of change is to be observed particularly well in cells of the accessory fifth motor nuclei and in the Gasserian ganglia where it is usual to find large bodies rather than small ones.

There is another type of alteration in the neurofibrillar substance clearly disclosed by this method and more commonly observed in the two above-mentioned groups of ganglion cells. That is the so-called "Alzheimer Fibrillenerkrankung" [23] in which coarse fibers and sickle-shaped bodies sharply stained by fuchsin appear within the cytoplasm. This change has been observed in other conditions and is thought by Spielmeyer [24] to be caused by a fusion of fibrils. In our preparations such structures have been found both in a stained and unstained condition. It is believed that they may be preëxisting in certain cells perhaps as an abnormality in the differentiation of neurofibrils and under the influence of rabies may become stainable by the fuchsin method.

It is thus demonstrated that under certain conditions in rabies the neurofibrillar material may be converted into bodies identical with "lyssa bodies." Whether this change is a specific one for rabies, that is, can occur only in the presence of the virus, it is impossible to say with the evidence at hand. However, there are indications that it may not be specific. For example, the bodies described by Lentz in the brains of dogs dead of *Staupe*, occurring both intracellularly and extracellularly, bear a striking resemblance to those formed in the last stages in the disintegration of the neurofibrillar substance which we have described. The size, shape, homogeneous structure and staining property are apparently identical. It seems possible that the extracellular situation of the *Staupe* bodies may be apparent rather than real, for when axis-cylinders and dendritic processes undergo the transformation described in rabbits the bodies sometimes appear to lie free in the tissue. Without careful study one might not associate them with cellular branches far from the

mother cell. It seems possible also that a similar change in the neurofibrillar material may give rise to the bodies which occur in ganglion cells of fowls infected with *Hünnerpest*, described by Kleine [25] and by Schiffmann [26] and suggesting to them a striking resemblance to Negri bodies.

The origin of "lyssa bodies" and the eosin-staining material of Negri bodies from an alteration in the neurofibrillar material seems to us very probable. Such a transformation into hyaline masses as has been described does not explain the architecture of typical Negri bodies nor account for the presence of "Innenkörper" which, in the opinion of many, constitute the essential part of these structures. One naturally asks whether the architecture of the typical Negri body and the nature of its inner structure are sufficiently characteristic and specific to enable one to disassociate them completely from the above-described degeneration products of the neurofibrillar substance and to continue to consider them perhaps as masses of the virus of rabies; or whether, admitting that the eosin-staining material may be composed of a substance originating from a cellular constituent (Babes), to regard the "Innenkörper" as representing the virus.

In considering this phase of the subject it is necessary to mention the description by Babes and by Koch and Rissling [27] of minute bodies, distinct from the bodies of Negri, which these authors regard as the etiologic agent of rabies. Babes found these structures only in the cytoplasm of ganglion cells, while the bodies of Koch and Rissling were found most numerously outside the cells. Judging from their descriptions and illustrations, the bodies found within the cells by both observers are possibly the same. Since our preparations have not shown any extracellular structures which may be considered identical with those occurring in the cytoplasm, the description of the bodies by Babes and his interpretation of them and their relation to rabies and Negri bodies will be adhered to in considering this phase of the problem.

Babes found in the cytoplasm of ganglion cells in regions where Negri bodies were few or altogether undemonstrable, minute bodies measuring about 0.1 micron and often occurring in pairs characteristically situated within a small vacuole. These bodies were best demonstrated by counterstaining a Ramon y Cajal preparation with Giemsa's stain, by which means they were colored almost

black and sharply exhibited. By the same procedure "Innenkörper" of Negri bodies were similarly stained, but they were often larger and more irregular in form. Babes was therefore of the opinion that he had demonstrated in the form of minute bodies lying in the cytoplasm within circumscribed spaces or vacuoles, the true etiologic agent of rabies. He interpreted the formation of Negri bodies as the product of a reaction on the part of the cell by which the virus became encapsulated, and the irregularities in size and shape of the "Innenkörper" as due to disintegrative changes going on in the imprisoned virus.

In our preparations from the brains of rabbits infected with rabies, bodies which we believe are identical with those described by Babes have been clearly demonstrated not only in ganglion cells but within axis-cylinders. It has been possible to throw some light, it is believed, on their origin and their relation to the formation of typical Negri bodies.

A study of the mitochondrial elements in ganglion cells and their processes and alterations of them under the influence of rabies, has enabled us to formulate the opinion that the small bodies of Babes and the architecture and content of typical Negri bodies are the result of disintegrative changes within the cell; and that they bear no other relation to the virus than that of an expression of its injurious action upon the cellular constituents.

The mitochondria of ganglion cells have not yet been sufficiently studied in their normal relations to offer a completely satisfactory cytologic background for accurate interpretation of their behavior under pathologic conditions. Their presence in normal, adult ganglion cells has even been denied by Meves, Duesberg and Hoven.[28] On the other hand Cowdry,[29] Held [30] and Spaltz [31] have been able to demonstrate them quite clearly in the body of ganglion cells, and they state also that similar structures occur along the axis-cylinders and dendritic processes, especially numerous about the end-organs of motor nerves.

It has been possible in our preparations to demonstrate structures resembling mitochondria in various types of ganglion cells. Filaments can also be demonstrated throughout the extent of axis-cylinders, corresponding in every way with those inside the cell, excepting that they are often much longer assuming the appearance of delicate fibrils of considerable length. These fibrils are, however,

not continuous and are therefore distinguishable from neurofibrils which have not been stained by the methods used. They seem in rabies to have a greater resistance to chemical injury than mitochondria are ordinarily considered to have, in that they are not destroyed by the acetic acid in Zenker's solution. Cowdry states that the mitochondria of nerve cells are destroyed by fixation in this fluid, but this has not been our experience either with normal tissue or that from rabid animals. It is true, however, that the mitochondria are ordinarily more difficult to demonstrate especially in axis-cylinders after fixation in Zenker's fluid. In the axis-cylinders from animals infected with rabies they seem to be more easily demonstrable and perhaps larger than under normal conditions. Within ganglion cells affected by the disease their number may appear greater than normal, but in later stages of the disease or in the presence of more profound injury they are greatly diminished and show various types of degenerative tranformation.

The method employed in this study especially for their demonstration in the central nervous system consisted in anesthetizing the animal with ether or chloroform, exsanguinating by cardiac puncture and fixing the brain immediately by injecting Helly's fluid through the carotid arteries. The brain was then removed and thin cross-sections (2 mm.) through the areas desired were cut and placed in the same fluid for twenty-four hours. They were removed to fresh Zenker's fluid without acetic acid, in which they remained three days; then they were washed in running water twenty-four hours and embedded in paraffin in the usual way. Sections 2 to 4 microns thick were stained by the acid fuchsin-methyl green method, or by the following technic which often gave better results with the medullated axis-cylinders:

1. Stain sections 1 minute in the carbol anilin fuchsin solution.
2. Wash in running water 1 minute.
3. Decolorize in a 1 per cent solution of acetic acid in 40 per cent formaldehyde.
4. Wash in running water 1 minute.
5. Counterstain in a saturated aqueous solution of picric acid 1 minute.
6. Wash in running water 1 minute, and repeat the entire procedure from 1 to 5, several times (usually four) depending upon the depth of coloration desired.
7. Remove the sections from the final immersion in picric acid and dehydrate rapidly in absolute alcohol. Xylol; balsam.

This method has previously been used with success in demonstrating gram-negative bacteria in tissues after fixation in Zenker's

fluid. The mitochondria appear as dark blue rods, granules or filaments against a yellowish background. The Nissl substance is unstained; Negri bodies are unstained or are light brown; and myelin sheaths are blue.

By these methods mitochondria have been demonstrated, after fixation in Helly's fluid, in ganglion cells in the pons, medulla, cerebellum, cervical cord and Gasserian ganglia, as well as in medullated axis-cylinders. After fixation in Zenker's fluid and staining with acid fuchsin-methyl green, they were also demonstrated in cells of the Gasserian ganglia and in axis-cylinders.

Ordinarily tissues fixed in Zenker's fluid are not so good for the study of axis-cylinders which are often shrunken. In one animal (R 9), however, the mitochondrial filaments and the changes to be described in them were especially well exhibited. This will form the basis of the following description, in as much as the history of this case has been given above in full. In other rabbits whose tissues were fixed by injecting Helly's fluid through the carotid arteries, identical changes were observed.

In the serial sections through the pons, the vestibular ganglion on the right side was included so that it was possible to follow the bundle of axis-cylinders from the ganglion completely into the substance of the pons. The myelinated axis-cylinders both distal and proximal to the ganglion were very well preserved. Very thin sections (2 to 4 microns) through this region were stained by the acid fuchsin-methyl green method and the following changes were observed in the cells and their processes.

All of the axis-cylinders and their myelinated sheaths appear superficially normal. In many of the axis-cylinders the rather long, sometimes wavy but always interrupted filaments lying parallel along the longitudinal axis can be very clearly seen. They seem to present a normal appearance, although with normal tissues they have not been so clearly demonstrable after Zenker's fixation. In other axis-cylinders side by side with these one can detect irregular thickenings along the threads causing the filaments to be somewhat nodular. In still others a very striking change in the filaments has occurred in that they appear partly or completely replaced by spaces (or an unstained substance) of considerable size, many times the diameter of the fibril, and of round or oblong shape often with their longitudinal poles pointed. Sometimes these polar points lead off

into a remnant of the filament. Three or four such vacuoles may be found lying together along a longitudinal axis as if a single filament had been thus affected at three different points. The margins of these vacuoles stain red against the pink background of an otherwise apparently homogeneous axis-cylinder. On their inner surface one can often see red-stained, irregular thickenings, sometimes one alone, again two with one situated at each longitudinal pole and less frequently three or more (Fig. 5, *a* and *b*). In similar preparations counterstained with Loeffler's methylene blue instead of methyl green, these bodies are stained deep blue in contrast to the otherwise red outline of the vacuole (Fig. 5, *c*). In such a preparation 1 to 2 microns thick, one can frequently trace these structures lying within red-outlined vacuoles along an axis-cylinder into the cytoplasm of a ganglion cell. Within the body of the cell they are irregularly distributed and vary in number. There may be only four or five, or as many as twenty, sometimes more. Similar structures are found in ganglion cells and axis-cylinders within the pons.

Some of the smaller vacuoles in the axis-cylinders have a relatively thick red-staining margin, and one may find here and there also in the same axis-cylinder an occasional independent mass or granule which corresponds to the thickenings observed in the more normal filaments. Stained by Mann's method the vacuoles are quite distinctly outlined by the blue stained material of the axis-cylinder. The margins may take a faint eosin coloration, but the granular bodies within are never distinct. In the nerve cells of the ganglion in which these structures appear, there are also many "lyssa bodies" of various sizes but all small. For the most part, these bodies closely approximate in size the small masses found in the vacuoles, the latter being somewhat smaller and more numerous. In the acid fuchsin-methyl green or methylene blue preparations, the two forms cannot be easily distinguished from each other unless the small bodies are seen in a distinct vacuole. The "lyssa bodies" appear as homogeneous, spherical, minute, red-staining structures irregularly arranged within the bluish cytoplasm. With the Mann or the carbol anilin fuchsin methods the small bodies of the type occurring in the axis-cylinder do not stain, while the "lyssa bodies" are demonstrable.

Identical changes in axis-cylinders especially of the fifth cranial nerves have been demonstrated in the brain and extradurally in

rabbits infected with rabies, after injecting the brain with Helly's fluid. It is often necessary to remove the chrome salts with potassium permanganate and oxalic acid before applying the acid fuchsin-methyl green or methylene blue methods, as a precipitate is otherwise apt to occur in the sections.

Such changes have not been found in the mitochondria of nerves and cells of four normal animals, in two of which the brains were fixed in Zenker's and the others in Helly's fluid. There is no indication that the changes are artifacts or the result of postmortem influences. In every instance in this study, only perfectly fresh material was used, all the animals having been killed and the tissues fixed immediately. Sections of the sciatic nerve proximal and distal to the cut ends and fixed in Zenker's fluid were studied by the same methods two and four days after section of the nerve. No similar degenerative change is observed. The axis-cylinders in these experiments underwent rather a granular disintegration, and fine fuchsinophile granules, which are interpreted as representing the remains of mitochondrial material, appear in the fragments.

One may safely conclude, it is believed, that the swelling and degeneration of the mitochondrial filaments in the axis-cylinder and presumably of similar filaments in the body of the cell are the result of the action of the virus of rabies on the living cell and its processes. It is also believed that this degeneration occurs with the formation of droplets of fluid indicated by the presence of the vacuoles, and of the small bodies within. None of the ganglion cells in which these bodies appear shows evidences of necrosis. The Nissl substance while pale is distinctly stainable and the nuclei are intact.

Mitochondria, it is true, are very susceptible to influences of an injurious nature. One finds frequently under various conditions ring-forms possibly resulting from an absorption of fluid in the process of disintegration, but never before have we observed or seen described the occurrence within mitochondrial rings of stainable material analogous to the bodies here described. Thus far the change appears to be specific. Whether these inner bodies represent transformation products of the filaments themselves or are the result of changes in the surrounding material is not clear, but the former explanation seems the more probable. They are regarded as degenerative in origin rather than as the virus of rabies. Babes thinks the virus is represented by the bodies described by him, with which, it is

believed, these inner structures are identical. We do believe with Babes, however, that they play a part in the formation of typical Negri bodies and minute "lyssa bodies." The occurrence within the same cell of minute forms of "lyssa bodies" comparable in size, number and distribution to the inner bodies as found in the vacuoles, suggests that the inner bodies may be transformed into "lyssa bodies." This takes place perhaps by a change in the surrounding neurofibrillar material so that it adheres to them and imparts an eosinophilic property. Also the individual vacuolated forms of Negri bodies are comparable to the small vacuolated structures which result from mitochondrial disintegration and which might, by a similar change in the neurofibrillar material, be transformed into vacuoles with an eosin-staining wall. For example, if degenerating neurofibrillar substance became swollen, fused and eosin-stainable, as it has been shown to be capable of becoming, and cohered incorporating the vacuoles with their inner bodies, the picture of the typical Negri body would result. Similarly, the "lyssa body" is an expression of the same process but without the presence or demonstrability of the inner bodies. According to this hypothesis the larger vacuolated Negri bodies might be formed by a coalescence of a few or many of the smaller vacuolated spheres, and in like manner some of the larger "lyssa bodies" might result from a confluence of the smaller ones. Under these circumstances one might expect to find Negri bodies composed of a mixture of the two forms. As a matter of fact one not infrequently observes in the rabbit rather large, sometimes lobulated Negri bodies, one lobe of which may be completely homogeneous while the other is vacuolated.

We agree with Babes that the "Innenkörper" of Negri bodies are probably the incorporated inner structures of the free vacuoles, for the latter correspond to them in size and show a distinct tendency to stain basophilically.

Discussion

In this series of rabbits infected with a strain of street virus of rabies by injection into the right masseter muscle, one finds necrotic ganglion cells both in the pons and the Gasserian ganglia in which no Negri bodies or similar structures are demonstrable. In the same preparations one may find other ganglion cells and axis-

cylinders which are apparently necrotic and in which the entire neurofibrillar material of the cell and its processes becomes transformed into a substance identical in staining property, so far as tested, with the eosin-stainable material of Negri bodies.

In a complete series of preparations, it has been shown that these altered neurofibrils are further transformed by fragmentation and cohesion into bodies within the cell and its processes. These bodies in gross structure and staining property are identical with what has been termed for convenience the "lyssa bodies," that is to say, bodies in every way similar to Negri bodies excepting the absence of demonstrable vacuolation and "Innenkörper." In other ganglion cells "lyssa bodies" and Negri bodies may occur side by side within the cytoplasm, but in our experience the former have greatly exceeded the latter in number. It is believed they are in reality just as specific for rabies, though for practical reasons they should not be so regarded.

In the same preparations degenerative changes in mitochondrial filaments of axis-cylinders and of the cell body are found which form structures very strongly suggesting early stages in the formation of both the vacuolated Negri bodies and "lyssa bodies." Changes are also observed in the neurofibrillar material of the cell body which seem to lead to the formation of the larger, more or less irregular, non-vacuolated "lyssa bodies," by a process of swelling, melting together and final fusion and coherence, associated with an altered staining property. In other words, all the structural changes within the nerve cells and their processes associated with rabies appear to have a degenerative rather than a specific origin, in the sense of representing the virus itself. The typical Negri bodies seem to be specific in so far as their intracellular situation and morphologic configuration are characteristic.

It is believed that the virus of rabies has not been revealed by our present methods. It is probable that it often overwhelms a cell causing it to die quickly without the formation of any characteristic cytoplasmic structure. Again it may kill the cell more gradually so that the entire neurofibrillar material undergoes a change by which it becomes readily and intensely stainable by the carbol anilin fuchsin and other methods used for the demonstration of Negri bodies. This change eventually results in a coalescence of neurofibrils and a breaking up of their substance into bodies resembling

Negri bodies. It is believed that this alteration is preceded by a disintegration of mitochondria.

In a less severe grade of injury or within cells of a higher degree of resistance, the degeneration may be only partial, manifesting itself more gradually and affecting only certain portions of the living cell. It causes in a sense a necrobiosis with the production of structures which physically may be identical with those just described but which because of a more gradual formation are more characteristic in their architecture. These forms often constitute the true Negri bodies, and they originate in a degenerative change in mitochondria which gives rise to vacuoles with bodies within them, about which through a partial disintegration of neurofibrillar material a capsule is formed.

The structure in the cell and its processes which seems to be the most sensitive to the injurious effect of the virus is the mitochondrial substance. Characteristic degenerative changes have been observed in these filaments in axis-cylinders as well as in the bodies of cells which otherwise appear relatively little injured.

The presence of this degenerative change in axis-cylinders at a considerable distance from the brain and from the mother cell is of especial interest in view of a possible axis-cylinder transmission of the virus of rabies. The occurrence of degenerative changes in the mitochondria of axis-cylinders may be interpreted in two ways.

It may be the result of an injury to the mother cell whose consequently disordered metabolism is reflected outward along its processes. The mitochondria, being especially sensitive to influences of an injurious nature, may disintegrate perhaps throughout the entire extent of the axis-cylinder. This particular type of degeneration, however, so far as we know, has not been described as occurring under the influence of toxic or other disturbances of nerve cells. If this is the true interpretation, it gives one little insight into the nature or activity of the virus, for the injury to the cell may be toxic in nature and not due directly to the presence of the virus within it.

On the other hand one may conceive of the virus itself passing along the axis-cylinder causing as it goes a disintegration of mitochondrial material. When the mitochondria are thus affected, they swell and are apparently transformed partially or completely into a fluid medium. This medium is perhaps favorable to the growth of the virus which may thereby be enabled to advance rapidly from

one level to another as the continuous formation of this favorable medium proceeds toward or away from the mother cell.

The facts at hand warrant the acceptance of the latter hypothesis, for the relatively slight evidences of injury within the ganglion cells from which these axis-cylinders proceed does not seem to be against it. Such a theory is perhaps the best explanation at hand for the fact that in the central half of the axis-cylinder processes from the vestibular ganglion in R 9, many of the axis-cylinders show the change in staining and final transformation into the "lyssa bodies" above described. Nearer to the ganglion they appear normal or show only a disintegration of mitochondrial filaments, and at the same time the mother cells are relatively slightly affected. Thus through the action of the virus the peripheral portion of an axis-cylinder may be completely destroyed, indicating that the effect of the virus may progress from the periphery inward toward the cell rather than the reverse. Under injurious influences of a general nature it has been shown (Bielschowsky) that the neurofibrils of axis-cylinders are more resistant than those within the body of the cell.

Conclusions

1. It has been possible to infect rabbits with rabies fairly regularly by inoculating them with street virus into the right masseter muscle.
2. The majority of animals thus inoculated and permitted to live long enough to develop muscular symptoms showed evidences of paralysis first in the fore legs and more often on the side of the inoculation.
3. It is believed that the virus enters the central nervous system through nerves supplying the inoculated muscle.
4. A method of staining to demonstrate Negri bodies is described.
5. A distinction is drawn between Negri bodies and "lyssa bodies," based on structural differences. "Lyssa bodies" were much more numerous in these animals than typical Negri bodies.
6. Following an inoculation of the virus into the right masseter muscle, "lyssa bodies" or Negri bodies were always demonstrable in the right motor nucleus of the fifth cranial nerve, but also in the left nucleus and widely distributed in the central nervous system. The most severe lesions, however, were always found in the pons, medulla and cervical cord, and apparently more extensive on the

right side. Ammon's horns were usually only slightly affected. Lesions in the sensory division of the fifth cranial nerve and in the cells of the corresponding Gasserian ganglion suggest an axis-cylinder transmission of the virus.

7. A degenerative change in the neurofibrillar substance of ganglion cells and their processes is described which leads to the formation of bodies identical with "lyssa bodies" and with the eosinstainable substance of Negri bodies.

8. It is believed that "lyssa bodies "are formed directly from such an alteration of neurofibrillar material in a portion of the living cell, with or without the inclusion of inner structures.

9. Degenerative changes are described in the mitochondrial material of axis-cylinders and nerve cells leading to the formation of structures which closely resemble the "Innenkörper" of Negri bodies.

10. It is believed that the typical Negri bodies are formed within the living cell by a focal degeneration of neurofibrillar material which melts together and coagulates or coheres about one or more of the structures which result from mitochondrial degeneration. The inclusion of these bodies gives the characteristic architecture to Negri bodies which alone serves to distinguish them from "lyssa bodies."

11. The type of degeneration of mitochondrial substance in axis-cylinders and nerve cells is, so far as we are aware, specific for rabies, in that small bodies are formed which stain deeply with Loeffler's methylene blue after acid fuchsin.

12. It is believed that neither the virus of rabies nor a constant structural change associated with its presence has yet been demonstrated microscopically.

13. All the structural changes observed appear to be degenerative in origin.

14. The hypothesis is advanced that the degenerative changes observed in axis-cylinders are the result of the passage of the virus along these processes, and indicate its manner of extension from one focus to another. The material resulting from the degeneration of mitochondria may serve as a favorable medium for the growth and spread of the virus.

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EXPLANATION OF PLATES XCII–XCIII

FIG. 1. Ganglion cell showing neurofibrils stained by the carbol anilin fuchsin method. The fibrillar network is evidently retracted from the original cellular margin. This is the first stage in the alteration of neurofibrils leading to the formation of structures resembling Negri bodies.

FIG. 2. Ganglion cell showing the second stage in this process. Neurofibrils are partially fragmented within the cell body, but are still preserved in the processes. The cytoplasmic bodies are in this stage very small.

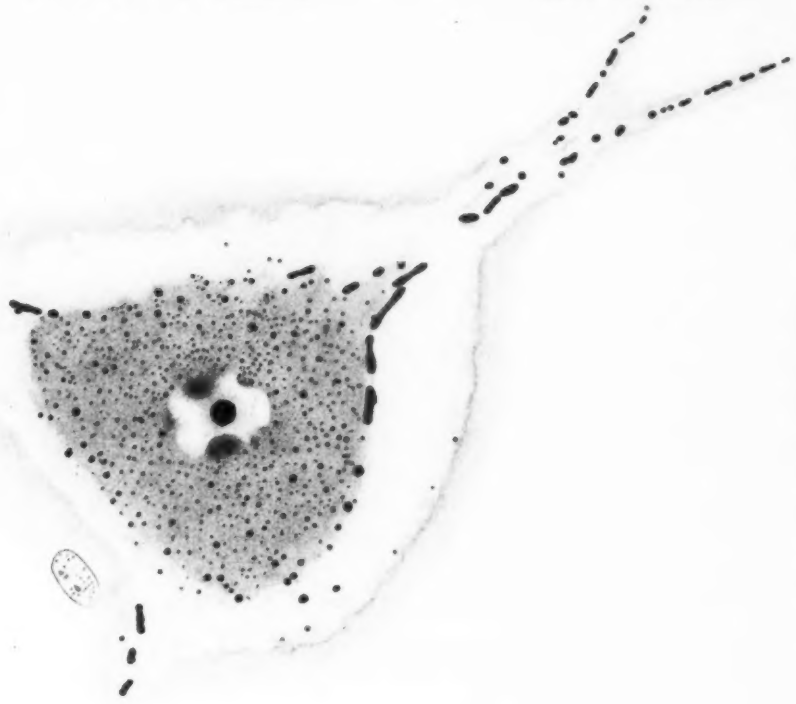
FIG. 3. Ganglion cell showing the final coherence and breaking up of the altered neurofibrillar material into bodies of various sizes identical with "lyssa bodies."

FIG. 4. An axis-cylinder showing a complete series of changes resulting in the formation of hyaline bodies. The hyaline structures lie within a delicate membrane. At the extreme left, the axis-cylinder is almost completely decolorized and appears like unaffected fibers.

FIG. 5. *a* and *b* are axis-cylinders stained by the acid fuchsin-methyl green method after fixation in Zenker's fluid. *c* is stained with acid fuchsin and Loeffler's methylene blue. In *a* one can see the mitochondrial filaments, showing areas staining more deeply. *b* shows the transformation of mitochondria into globules. One can see the irregular thickenings along the inner margins. *c* illustrates the reaction of these granules to the counterstaining with methylene blue. Such dark blue dots within vacuoles could be traced along axis-cylinders into the body of the cell.

1

2



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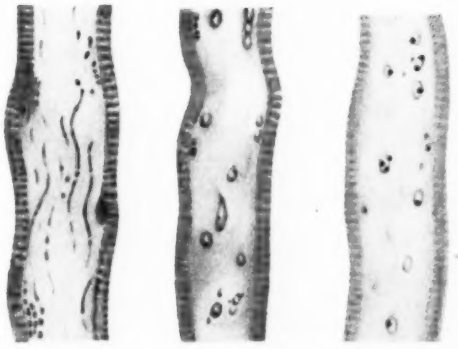

5a 5b 5c

Goodpasture Rabies and Negri Bodies

DIFFUSE TUMORS OF THE MENINGES*

WILLIAM BOYD, M.D., M.R.C.P. (EDIN.)

(From the Departments of Pathology of the University of Manitoba and the Winnipeg General Hospital)

One of the most remarkable examples of tumor formation is that known as diffuse sarcomatosis of the meninges. Elsewhere in the body, as implied by its very name, a tumor is made up of cells or fibers collected together to form a mass. That mass, it is true, may be flattened and widespread, as in endothelioma of the pleura or the peritoneum, but even there flat elevations or multiple nodules may be recognized.

In the leptomeninges, however, there occasionally occurs the curious condition of a neoplasm which does not give rise to a mass. A considerable number of cases of this disease have been described, but many interesting points in the pathology of the condition still remain to be cleared up.

Such terms as diffuse sarcomatosis of the meninges are somewhat misleading for they give one the conception of a pathologic entity which does not really exist. There are indeed three ways in which the condition may arise: (1) it may be secondary to some tumor outside the central nervous system; (2) it may be secondary to a tumor originating in the central nervous system; or (3) it may (possibly) exist as a primary lesion of the meninges.

Many forms of neoplasm may occasionally set up metastases in the brain, and those most prone to do so are the most likely to give rise to diffuse involvement of the meninges. The tumor may be a carcinoma or a sarcoma. Judging from the recorded cases the latter is much the more common, although it is more than probable that very many of the cases of sarcoma have no real claim to be included in this class of neoplasm. Carcinoma of the lung is perhaps the form of cancer most frequently associated with cerebral metastases. In 105 cases of carcinoma of the lung Dosquet [1] found metastases in the central nervous system in 31.4 per cent. Morse [2] has recently described an interesting case of diffuse involvement of the meninges

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in which the primary tumor was an unsuspected carcinoma of the lung only discovered at necropsy. In this and in the other instances where diffuse meningeal involvement was observed, there was a definite tumor of the brain which, as will presently be seen, probably constituted the focus from which the meninges were involved. Although the relative incidence of cerebral metastases is not so high in cancer of the stomach and of the breast, yet these diseases on account of their great frequency are those most likely to be responsible for secondary growths in the brain.

Before discussing the various forms of sarcoma which may be found in a diffuse form in the cerebral meninges and the paths by which the tumor cells gain access to the subarachnoid space, it will be convenient to present the details of a case which recently came under my observation.

Clinical History

The patient was a Galician, 41 years of age, who commenced to suffer from headaches about the middle of December, 1923. The pain came on every few hours and lasted for from ten to twenty minutes. As the headaches increased in frequency and in severity he came to the hospital on December 19th. The pain at the beginning of an attack was first felt in the forehead, but presently it passed over the top of the head and down the back of the neck. Soon the pain became constant, but still much worse in spells during which he groaned and held his head between his hands. At no time did he complain of any dizziness or tinnitus.

On admission he appeared to be in a lethargic condition, manifesting a listlessness which increased in intensity so that he lay quietly as if asleep most of the time. He could be easily aroused, however, and then answered questions quite intelligently. At this time a provisional diagnosis of encephalitis lethargica was made. The cranial nerves appeared to be normal. The eyes reacted to light and accommodation. There was no optic neuritis. The deep reflexes were unimpaired. The plantar reflex was flexor in type. The abdominal and cremasteric reflexes were present. Owing to the mental condition it was not possible to make a satisfactory examination of the sensory functions.

On the back and the anterior abdominal wall there were several small, firm, painless, non-pigmented nodules. In the right lumbar region there was a pedunculated fungating mass about 2 cm. in diameter, which had apparently been present for a number of months and which had increased considerably in size during the last few weeks.

Soon after admission to the hospital he developed stiffness of the neck and a double Kernig's sign. A lumbar puncture was done in order to exclude the possibility of syphilitic meningitis. The spinal fluid, which was not under increased pressure, was clear, showed a marked increase in globulin and contained 280 cells per c.mm. The Wassermann test was negative both in the blood and in the spinal fluid. The cells in the spinal fluid were peculiar and quite unlike any

which I had seen hitherto. They were large and pale, with a large oval nucleus and abundant cytoplasm. The sugar in the fluid was 0.05 gm. per 100 c.c.

A portion of the fungating tumor of the skin was removed and was found to be a pigmented melanoma showing numerous mitotic figures and evidence of rapid growth. A diagnosis was then made of melanoma with cerebral metastases.

The lethargy increased and the headache became worse. On January 11th the patient developed a right-sided facial paralysis. He now lay in a semi-unconscious condition all the time, the head retracted and the knees drawn up. The plantar reflex showed an extensor response on the right side and occasionally a similar response on the left side. He commenced to vomit, gradually became completely unconscious and died on January 15th.

Necropsy Findings

The *large nodule in the skin* was removed and examined with greater thoroughness than had before been possible. Part of the nodule was deeply pigmented, even to the naked eye, but other parts were completely free from pigment. The tumor cells were large and spherical with very pale cytoplasm and an oval vesicular nucleus, were arranged in indefinite alveoli and could be seen to arise in the most unmistakable manner from the deepest layer of the epidermis. The other skin nodules were of similar structure, but they presented one important difference, namely, they were situated quite deep and separated from the epidermis by a considerable interval. It appears, therefore, that they were metastatic in origin, a point of considerable importance which will be referred to in the subsequent discussion.

The *thoracic and abdominal organs* were normal with the exception of the *left kidney* which presented a small circumscribed nodule in the cortex about 1 cm. in diameter. This nodule was composed of large spherical cells most of which were non-pigmented, but some of which were loaded with fine yellow granules. In the immediate neighborhood of the nodule the outline of several glomeruli packed with pigmented tumor cells could be made out. It appears certain, therefore, that emboli of tumor cells were being given off from the fungating nodule in the lumbar region and that some of these were arrested by the glomeruli of the kidney, while others formed secondary nodules in the deeper layers of the skin.

Brain. When the brain was removed in a somewhat poor light it seemed at first to be quite normal and certainly presented no gross tumor either to the sight or touch. Closer inspection in a good light, however, showed that there was a faint darkening of the pia-arach-

noid in certain regions and associated with the darkening there was a slight thickening. What appeared to be a thin film, yellowish brown and adherent to the underlying structures, covered the greater part of the cerebrum, the brain stem and the cerebellum. The condition was most marked over the left cerebral hemisphere involving the frontal lobe as far back as the fissure of Rolando and commencing again in the occipital region; it was difficult to determine with accuracy the exact limitation. The left frontal lobe was involved to a less degree. A distinct thickening and opacity were found in the interpeduncular space and over the brain stem and cerebellum.

The brain was opened by the usual incisions but, as these did not actually pass through the gray matter, no abnormality was revealed. When, however, the cerebrum was sectioned from before backward in a series of coronal sections, a very remarkable condition at once became apparent. The actual tip of the frontal lobe appeared normal, but on passing back for 2 or 3 cm., the sections showed the pia to be not only discolored but slightly thickened. It was firmly adherent to the cortex and could not be stripped off. A change was observed at the same time in the gray matter of the cortex, which also became distinctly discolored, the gray passing into a dirty brown in striking contrast to the pure white of the unaffected white matter. At the same time it was noticed that the cortical gray matter was slightly thicker than normal, averaging from 2 to 3 mm. in width. This thickening was seen in much the most striking manner not in the superficial cortex but in the folds of the convolutions surrounding the fissures. Here the gray matter was two or three times the normal thickness and appeared to be the seat of some kind of infiltration, an appearance which was particularly striking in the occipital lobe. Both surfaces of the cerebellum showed darkening of the pia, patchy rather than uniform in its distribution.

Further examination of the cut surface under the low power dissecting microscope showed that the pigmentation did not really penetrate into the full depth of the gray matter but only for about one third the distance. The apparent expansion of the gray matter surrounding the sulci was due to an infiltration of the pial prolongations with cells, thus producing a widening of the fissure. The cells, however, were packed with material which blended with the gray matter and thus gave rise to an appearance of spurious thickening.

The ventricles were not dilated nor did any part of their lining show tumor nodules. The choroid plexuses seemed normal. No tumors were found in any part of the brain substance.

The spinal cord was clothed with the same thin yellowish brown film which covered the brain. There did not appear to be an invasion of the cord itself.

Microscopic Examination of the Brain

Under the microscope it was at once seen that the discoloration of the surface of the brain was due to the fact that the subarachnoid space was filled with cells which infiltrated every nook and cranny of that space. It was as if molten wax had been poured into the space and had then been allowed to solidify. The cells were of the same type as those which constituted the skin tumors except that the nucleus as a rule was darker and more eccentric in position. A more striking difference lay in the fact that none of the cells was pigmented. Just as the discoloration of the surface varied in intensity in different places, so the degree of infiltration of the subarachnoid space varied in a corresponding manner. Although the picture in its general design was that which one associates with acute meningitis rather than cerebral tumor, yet there could not be the slightest doubt that the cells were tumor cells which, because of the special anatomic arrangements, spread out in a diffuse manner instead of forming a neoplasm.

In the cerebral cortex there were numerous masses of cells which at first sight appeared to be completely separated from those in the subarachnoid space and which were so markedly perivascular in their grouping as to suggest that they were derived from the vascular endothelium (Figs. 1 and 2). In occasional fortunate sections, however, it was clearly seen that these perivascular sleeves accompanied the vessels passing from the meninges into the cortex and that the cells were directly continuous with those in the subarachnoid space. That is to say, the infiltration of tumor cells had flowed along the perivascular prolongations of the subarachnoid space for a variable distance into the brain substance (Figs. 3 and 4). A remarkable feature of these perivascular masses was that the cells in many cases were loaded with yellow pigment, although those in the adjoining subarachnoid space were completely free from pigment.

This may possibly be accounted for by the more advanced age of the deeper cells.

The walls of both lateral ventricles (thalamus and caudate nucleus) were carefully examined but no tumor cells or any implants on the ependyma could be found. A number of large cells were observed in the choroid plexuses of the lateral ventricles. It was not possible, however, to decide whether these should be regarded as tumor cells.

Tumor cells were present in the subarachnoid space and in the subjacent brain tissue of the midbrain, pons and medulla. The cranial nerves issuing from these parts of the brain carried with them a sheath of tumor cells which penetrated between the main bundles of fibers.

The meninges covering the cerebellum were infiltrated with tumor cells but none was observed in the cerebellar cortex. The same was true for the spinal cord which was surrounded by a sheath of cells particularly thick in the anterior and posterior fissures. The substance of the cord and the central canal were uninvolved.

Summing up the necropsy findings, we may say that there were collections of tumor cells of the melanoma type in the skin, in the kidney and in the subarachnoid space of the brain and spinal cord; that these cells were continued into the perivascular and perineural continuations of the subarachnoid space; and that the cells in these latter situations were pigmented, whereas those in the subarachnoid space were not.

Discussion

It has already been pointed out that diffuse infiltration of the meninges, the condition commonly known as diffuse sarcomatosis of the meninges, may arise in one of three ways. It may be primary in the meninges, it may be primary in the brain and secondary in the meninges and finally it may be primary elsewhere and secondary in the meninges with or without involvement of the brain. In the first instance the tumor is a melanoma, in the second it may be a glioma or a melanoma and in the third it may be a carcinoma, a sarcoma or a melanoma.

True primary sarcomatosis (melanosis) of the meninges is probably a condition of great rarity. The term, however, must not be used in a loose manner. In a recent communication on "Primary

Sarcomatosis of the Leptomeninges," Ford and Firor [3] give details of four cases. In the first of these there was a tumor in the right hippocampus, in the second there was a tumor in the fourth ventricle and in the remaining two no necropsy was performed. Under those circumstances one is hardly justified in speaking of the meningeal condition as being primary.

Even in the absence of a tumor in the brain, caution must be observed before concluding that the condition originated in the meninges. The eyes must first be examined for the possible presence of a melanoma. As Bland-Sutton [4] points out, while melanoma of the choroid usually occurs as a discrete tumor, it may occasionally assume the form of a diffuse flat infiltration the quiet growth of which makes detection difficult. Some cases of melanosis apparently primary in the meninges may thus in reality be secondary to unrecognized tumors in the eye.

When all these possibilities have been excluded there still remains a small group of cases in which the condition appears to have originated in the meninges. The pia, especially that portion which covers the anterior surface of the medulla, contains many pigmented cells. In the negro, indeed, this part of the brain may present a darkening which can be recognized by the naked eye. From these cells a primary melanoma may take its origin.

While the possibility of a primary meningeal origin of these tumors must be admitted, it should not yet be regarded as proved. The anatomic findings in the supposed primary cases are exactly duplicated in cases which are undoubtedly secondary. This is well seen in a remarkable case recently reported by Weller [5] in which diffuse melanotic involvement of the meninges was associated with a recurrent melanosarcoma of the skin. In this case the body was simply riddled with metastases, nodules being found in the lungs, spleen, liver, kidneys, adrenals, bladder, prostate, testes, pancreas, intestinal wall and all the lymph glands, while the myocardium was studded with melanotic nodules. Melanomas were present in the floor of the fourth ventricle and in the choroid plexus so that either of these sites, both of which are exposed to the stream of cerebrospinal fluid, may have served as the invading points for the meninges. Our own case serves as an intermediate link between Weller's case and those of the supposed primary melanoses of the meninges.

There still remains to be considered a group of cases in which

melanosis of the meninges is associated with melanomas of the skin, but in which the two conditions are supposed to be quite independent. Rokitansky,[6] Oberndorfer,[7] Grahl [8] and Maclachlan [9] have described cases of extensive pigmentation of the brain associated with pigmented nevi in the skin. The cerebral pigmentation was confined to the meninges, the surface of the brain and the lining of the ventricles. The pigmented cells in the cerebral cortex showed a marked perivascular arrangement. None of these authors considers that the pigmentation of the brain was in any way secondary to the pigmented nevi in the skin. Berblinger [10] in a very careful study comes to the same conclusion. The title of his paper, "Multiple Melanomata of the Skin with Neurofibromatosis of the Cutaneous Nerves, Melanotic Tumor of the Cerebrum, Glioma of the Pons, Sarcomatosis of the Meninges, and High Grade Congenital Hydrocephalus in a Child of Nine Months," summarizes the pathologic lesions found. He considers that two separate processes were at work: (1) melanomas of the skin and (2) melanoma of the brain which was secondary to a primary melanosis of the membranes. The perivascular arrangement of the tumor cells in the cerebral cortex was identical with that of our own case.

The principal reason why Berblinger refuses to admit that the tumor in the meninges can have any relation to those in the skin is that no other secondary growths were found in the rest of the body. To this, two points may be raised in answer. First, Ribbert has shown that melanomas may metastasize while as yet the primary nodule shows no sign of malignancy. And second, if in our own case the nodule in the kidney which was undoubtedly metastatic in nature had either been overlooked or had not been present, the same argument could have been urged against a causal relationship between the meningeal and skin lesions; whereas there cannot be the slightest doubt that the one was directly dependent on the other. The matter is not susceptible of direct proof, but it appears much more reasonable to suppose that with occasional connecting links such as the metastatic nodule in the kidney the two conditions are related and not separate and distinct.

The second group of cases is that in which a primary tumor of the brain is associated with secondary diffuse involvement of the meninges. There is no occasion to say much on this subject. In all the cases we have found described in the literature, the primary tumor

has been situated in some part of the brain with direct access to the cerebrospinal fluid, usually in the wall of the third ventricle or the floor of the fourth ventricle. The tumor erupts into the subarachnoid space just as a carcinoma of the stomach or the ovary may erupt into the peritoneal cavity and scatter its cells broadcast throughout that sac. Small implantation growths may occur on the lining of the ventricles, as in a case described by Stanley Barnes,[11] in which a tumor of the frontal region had ruptured into the lateral ventricle and was associated with several small soft masses scattered throughout the lateral, third and fourth ventricles and the aqueduct of Sylvius.

With regard to those cases of evident metastases to the meninges from primary tumors situated outside the brain or spinal cord, sufficient reference has already been made in the opening pages of this paper. Although carcinoma, sarcoma and melanoma are the principal tumors recorded in the literature, there appears to be no reason why any type of malignant new growth should not behave in this manner.

Cells in the cerebrospinal fluid. In many cases of diffuse tumors of the meninges, including our own, peculiar cells have been found in the cerebrospinal fluid. These cells, sometimes of great size, may be multinucleated and even show mitotic figures. Usually they are taken for large lymphocytes or an endothelial type of cell. The cytoplasm is clear, often vacuolated and the nuclei are large and strongly basophilic. Some excellent figures of these cells appear in an article by Chatelin [12] in the Nelson Loose-Leaf Medicine. These cells must be regarded as true tumor cells. The fact that in melanomas they do not contain pigment must not be taken as an argument against their neoplastic nature, for we have already seen that the tumor cells in the subarachnoid space may be quite free from pigment as in our own case.

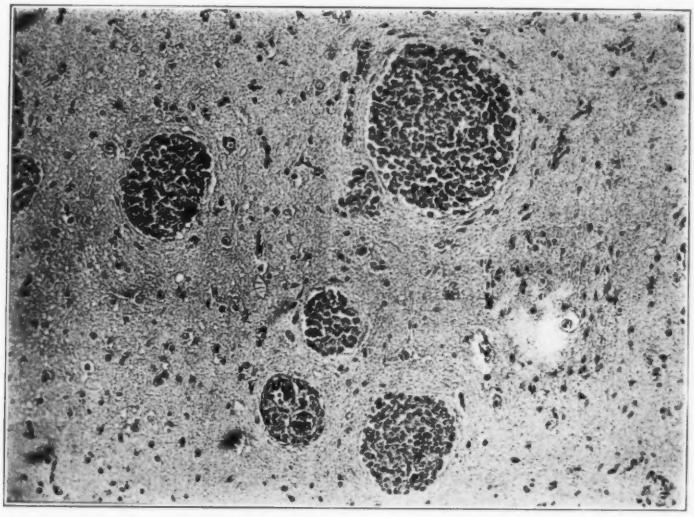
In conclusion, we may inquire what is the mechanism by which the tumor cells are distributed in such a case as that which has been described in this paper. It must at once be apparent that the cerebrospinal fluid is vitally concerned with this spread. Not only is the subarachnoid space both of the brain and of the spinal cord filled with tumor cells; not only are the primary tumors always situated in parts of the brain or cord readily accessible to the fluid; not only do the implantation growths occur solely in those parts which are

bathed by the fluid; but even the uttermost bounds of the subarachnoid space, even its farthest extensions along the vessels which penetrate into the brain and along the nerves which leave it, are permeated by the tumor cells. This permeation appears to be of a passive rather than an active character, for we could find little or no evidence of actual invasion of the brain substance. It is as if the tumor cells had been poured into the subarachnoid space as into a mould and had set there in the form of a jelly.

Both the gross and the microscopic appearance remind one of the picture of meningeal inflammation. Oppenheim[13] indeed speaks of a diffuse meningeal process rather than a true tumor formation. Lewkowicz[14] of Warsaw has pointed out in a recent paper that the prevailing views regarding meningeal infection fail to satisfy the needs of the case and that it is highly probable that instead of the infection commencing in the meninges it really originates in the choroid plexus as an ependymitis. The inflammatory cells, he believes, are carried by the flow of cerebrospinal fluid from the ventricles outward to the subarachnoid space and upward to that part of it in relation to the vertex of the brain. Some such mechanism may also be at work in cases of diffuse sarcomatosis of the meninges. From a focus in the choroid plexus, or in the case of a primary brain tumor in the cerebral substance, the tumor cells are carried outward by the flow of the cerebrospinal fluid and passively fill the subarachnoid space and all its ramifications.

Summary

Diffuse infiltration of the meninges with tumor cells, commonly known as diffuse sarcomatosis of the meninges, is in the great majority of cases a secondary condition. While the possibility of a primary meningeal origin must be admitted, this possibility is becoming open to doubt. The mechanism of the meningeal involvement is invasion of the cerebrospinal fluid with tumor cells from some focus standing in intimate relation to the fluid, such as the choroid plexus or the wall of the ventricles. A clinical diagnosis of the condition may be made from the discovery in the spinal fluid of large pale cells in considerable numbers.



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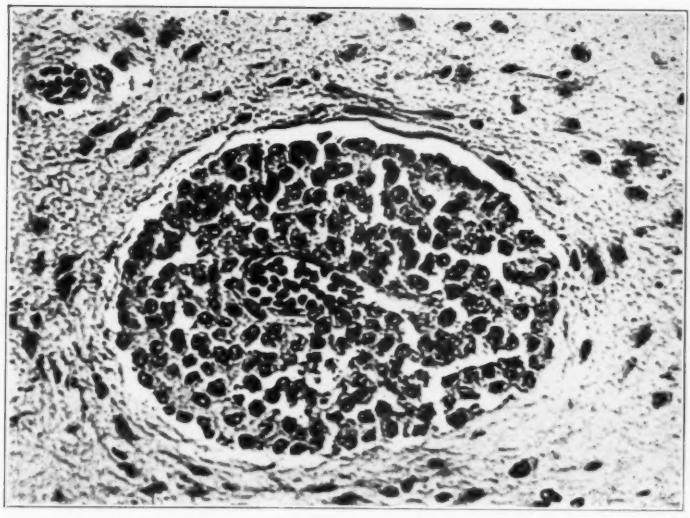

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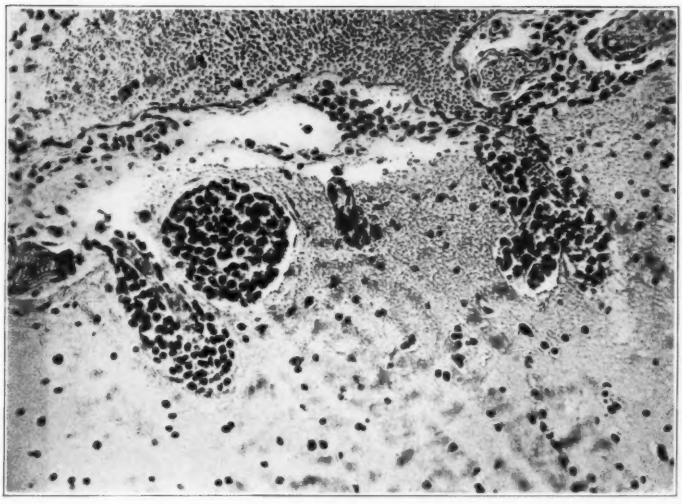

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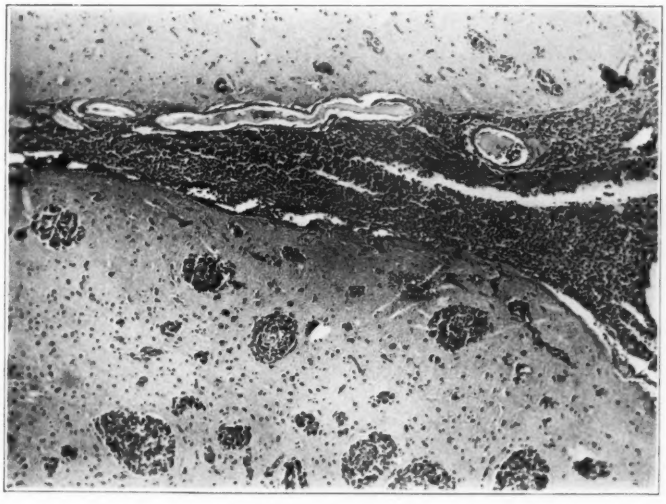

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DESCRIPTION OF PLATES XCIV–XCV

FIG. 1. Groups of tumor cells scattered throughout the cerebral cortex and apparently not connected with the surface.

FIG. 2. Mass of tumor cells grouped around a small vessel.

FIG. 3. Showing the true method of formation of the tumor masses by extension of the cells along the perivascular prolongations of the subarachnoid space into the substance of the brain.

FIG. 4. The meninges in the fissures are infiltrated with tumor cells.

SPLENOMEGALY (TYPE GAUCHER) AND LIPOID-HISTIOCYTOSIS (TYPE NIEMANN) *

WILLIAM BLOOM

(From the Snydacker Fund of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research, Chicago)

Since the description by Gaucher [1] in 1882 of an unusual symptom complex associated with large cell splenomegaly, numerous reports of supposedly similar cases have appeared. The diagnosis of a few of these has occasioned much controversy and the nature of some of them has not yet been settled. According to Pick [2] there are in the literature thirty-two well authenticated cases of Gaucher's disease; eighteen of these were studied at necropsy and in fourteen the surgical removal of the spleen yielded material for study. We have had the opportunity of studying material from two cases of Gaucher's disease and from three cases of a condition which until recently has been considered by some as Gaucher's disease. In a very recent exhaustive study of splenomegaly of the Gaucher type by Epstein [3] several cases of the condition which is certainly not Gaucher's disease are still counted as examples of it. We are, therefore, making this report to emphasize the striking differences between Gaucher's disease and this other condition and to attempt to throw some light on the nature of the latter.

GAUCHER'S DISEASE

The clinical and anatomic features of Gaucher's disease are so constant and have been recorded so frequently in recent articles that it is unnecessary to consider them in any detail here. Clinically it is characterized by its marked chronicity, leucopenia, mild secondary anemia, slight tendency towards bleeding, a peculiar brown-yellow pigmentation of the skin, splenomegaly and cirrhosis of the liver without ascites. Anatomically one finds in the liver, spleen, lymph nodes and bone marrow peculiar large pale cells which have been ascribed to various sources. Gaucher described the disease as an "épithéliome primitif de la rate." Cornil [4] thought the large

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cells in this case to be derived from reticulum cells. In the opinion of various authors since then, these cells arise from lymphocytes, endothelium or reticulum cells alone, or from both reticulum cells and endothelium. The careful and extensive studies on this subject made by Risel,[5] Mandlebaum[6] and Pick indicate that the Gaucher cells are reticulum cells and possibly certain endothelial cells which have stored large amounts of a substance which, according to the chemical investigation of Epstein,[7] seems to be closely allied to cerebrin.

The Gaucher cells with certain stains, especially with Mallory's aniline blue connective tissue stain, show a distinct longitudinal striation of the cytoplasm. They frequently give a positive iron reaction with the Turnbull blue method. None of the lipoid staining reactions is typical. After mordanting in potassium bichromate there is a very pale yellow or blue-staining of these cells with Sudan III or Nile blue. Anisotropic bodies are seen but very rarely in them.

The morphologic and histochemical reactions mentioned in the above paragraph are so constant in a very large percentage of the cases reported as examples of Gaucher's disease that they have been suggested as the criteria to be applied to all cases resembling this disease. They are evident in characteristic fashion in two cases reported herewith.

Report of Two Cases of Gaucher's Disease

Case I. R. M., No. 61854, a Jewish boy aged 6 years was admitted January 13, 1925, to the surgical service of Dr. A. A. Strauss with the complaints of enlarged abdomen, vomiting and nose bleeds. Family history and past history were negative. Three older brothers were quite healthy.

The parents of the child "always knew that the abdomen was enlarged and paid no attention to it." A physician, who was called to see the child because of a hemorrhage from the nose, found an enlarged spleen. Nose bleeds had occurred every two or three weeks for the past three years. During the month before admission the child received Fowler's solution. In this period there was slight bleeding from the nose every day. The appetite had always been good. No blood was ever seen in the stools and no petechiae in the skin.

On examination the skin was quite pale. The sclerae had a slight bluish tinge. The examination was otherwise negative except for the abdomen. This was enormously enlarged, soft and symmetrical. The spleen extended to the iliac crest in the left axillary line and below the umbilicus in the midline. It was smooth and not tender; the notch was felt near the umbilicus. The liver extended one hand's breadth beneath the costal margin and was smooth, firm and painless. There was no evidence of fluid in the abdominal cavity.

The blood on admission, Jan. 14, 1925, contained hemoglobin 53 per cent; red blood cells 2,810,000 and white blood cells 1,550 per c.mm.; neutrophiles 34 per cent; small mononuclears 56 per cent; large mononuclears 4 per cent; transitionals 4 per cent; eosinophiles 1 per cent. The Wassermann reaction was negative. On January 17th, hemoglobin 50 per cent; red blood cells 2,730,000 and white blood cells 2,050 per c.mm.; fragility complete, 0.32 per cent NaCl, and partial, 0.4 per cent NaCl. The indirect van den Bergh reaction was within normal limits; the direct was negative. The urine was negative for bile pigment and urobilin.

The child was given a transfusion of 200 c.c. of blood which was followed by splenectomy on January 20th. He improved after the operation. The blood on February 4th showed: hemoglobin 70 per cent; red blood cells 3,090,000 and white blood cells 3,400 per c.mm.; neutrophiles 42 per cent; small mononuclears 52 per cent; large mononuclears 5 per cent; eosinophiles 1 per cent.

Gross Examination: The spleen measures 19 × 12.5 × 8 cm., weighs 650 gm. and is reddish orange. The capsule is smooth and slightly thickened. On section there are many translucent grayish areas surrounded by thin red zones. They are circular, about 0.5 to 1 mm. in diameter and are probably Malpighian bodies. The rest of the pulp has a peculiar grayish orange, granular appearance. The spleen is somewhat soft. Attached to the hilum of the spleen are several medium sized lymph nodes which are soft and light gray-yellow on section. Beneath the capsules of two of these nodes are a few pin-point-sized gray-white dots. The liver is greatly enlarged, irregular in outline, uneven of surface and grayish brown. A small portion is removed at operation. There is no ascites.

Microscopic Examination: An unstained frozen section of the spleen freshly fixed in 10 per cent formalin shows most of the spleen to consist of nests of large pale cells. These are round, oval and at times fusiform in shape. The cytoplasm is composed of a hyaline homogeneous gray-white substance. In paraffin sections stained with hemalum and eosin (Fig. 1) most of the sinuses and the greater portion of the pulp are filled with nests of these large pale cells. The nuclei are fairly large, but are small when compared with the cytoplasm which in places measures 50 to 60 micra in diameter. Some of the cells contain three and four nuclei but the majority are mononucleated. The nuclei have but small amounts of chromatin material. The cytoplasm of these cells stains diffusely but only faintly with eosin. The walls of most of the sinuses are slightly thicker than normal. Some of them are lined with the large cells; in others the sinuses are completely filled with them. In some of the sinuses many red blood cells lie between the large cells and it is usually in

such places that the latter have taken up and are stained dark brown with granules of brown pigment. The few Malpighian bodies which are present are surrounded by fairly large zones of erythrocytes. A few of the follicles contain some of the large cells in the germinal centers. With Mallory's aniline blue the large cells, for the most part, are distinctly spindle-shaped; their outlines are quite indistinct and the cytoplasm contains numerous parallel wavy fibrils running in the long axis of the cells. The spaces between these fibrils are occupied by a pale blue substance. This appearance has been described in great detail by Epstein.

When stained with the Turnbull blue method for iron, most of the large cells take on a diffuse pale blue color. Under oil immersion the cytoplasm is traversed by wavy blue lines. A few of the cells are very deep blue; others have a few dark blue crystals in their cytoplasm. The diffusely dark brown, pigmented cells lose the pigment after treatment with ammonium sulphide which indicates that the pigment is a formalin precipitate. Frozen (formalin fixed) sections stained with Sudan III and counterstained with hemalum show an almost imperceptible, diffuse grayish yellow tinging of the cytoplasm of the large pale cells. In sections stained with Nile blue sulphate the large cells have a diffuse, light blue color of about the same intensity as the rest of the cells in the section. Frozen sections stained by the Lorrain Smith-Dietrich method show a light brown and in places suggestively gray cytoplasm in the large cells. The resultant color depends largely on the degree of differentiation. Certainly the reaction can hardly be considered positive. After mordanting in potassium bichromate the large cells stain a definite though very faint yellow with Sudan III and a very pale blue with Nile blue. The staining by this method is diffusely even throughout the cytoplasm. Although it is very faint, the reaction must be considered positive. In frozen sections stained with Weigert's iron hematoxylin one sees great clumps of pale-staining large cells. These stain a homogeneous bluish gray and the nuclei are darker than the cytoplasm, of which the color is approximately the same as that of the smooth muscle of the vessels. The color of the cytoplasm is somewhat darker than that of the collagen but nowhere as dark as the nuclei.

In the lymph nodes, beneath the capsule, are pale giant cells occurring singly and in small groups. They are usually placed radially

in the nodes. The cells here are quite long and with the Bielschowsky method each cell is surrounded by fine strands of reticulum fibrils. In the section of liver there is a marked increase in the periportal connective tissue. With the Mallory aniline blue stain (Fig. 2) this connective tissue is heavily infiltrated with the same large cells that are so numerous in the spleen. A few of these large cells are in the centers of the lobules. Here they have no connection with the liver cords or Kupffer cells; they appear rather to be in contact with the connective tissue about the efferent vein. This case both clinically and anatomically fulfills the criteria mentioned above for Gaucher's disease.

CASE II. Mrs. L. T., No. 13350, a Jewish woman, aged 42 years, was admitted Sept. 22, 1919, to the surgical service of Dr. A. A. Strauss. The complaints on admission were a mass in the left side of the abdomen, pain in the bladder region, weakness and slight loss of weight.

The patient had not had typhoid, malaria or pneumonia. The menstrual periods had been regular and normal until shortly before admission when they became irregular. There had been some bleeding between periods during the last two or three months. The family history was negative.

About nine years before admission, the patient first noticed in the upper left quadrant of the abdomen a small mass which grew progressively larger. There had never been any pain or discomfort connected with the mass. The pains in the bladder region had been present only for the last two or three weeks. The patient described the condition as a sensation of pressure and not a real pain. The condition simulated a desire for urination. During the last two weeks urination had been as frequent as four or five times an hour. In the patient's opinion she had lost some weight but she did not know the definite amount.

Physical examination was negative except that the spleen was palpable. It extended from the costal margin to the iliac crest and to the umbilicus in the midline and was soft and smooth.

The stools and urine contained no abnormal constituents. Blood examination on Oct. 22, 1919 revealed hemoglobin 85 to 90 per cent; red blood cells 5,600,000 and white blood cells 6,800 per c.mm.; neutrophiles 61 per cent; small mononuclears 29 per cent; large mononuclears 6 per cent; transitionals 0 per cent; eosinophiles 2 per cent; and unclassified cells 2 per cent. The Wassermann reaction was negative.

Splenectomy was performed on Oct. 30, 1919.

Gross Examination: The spleen measures 19 × 13 × 5 cm., weighs 725 gm., and is quite firm. The surface is smooth, the capsule thin, and the color through the capsule brownish red. The cut surface is uniformly brownish red, with little blood. The connective tissue is not increased and the lymphoid tissue is not visible. An accessory spleen, 1.5 cm. in diameter, is of the same appearance and is firm on section.

Microscopic Examination: The spleen is almost a replica of the one described above. The type and arrangement of the large pale cells is practically the same in both. There is, however, slightly more free splenic pulp in this case. The cells with Mallory's aniline blue stain show the typical longitudinal striations which are characteristic of Gaucher cells. The cells give a very slight pale blue stain with the Turnbull blue reaction for iron. They stain very faintly with Nile blue and Sudan III after mordanting in potassium bichromate. The Lorrain Smith-Dietrich and Fischler stains are negative, as are the Golodetsky and the iodine tests for cholesterol. The accumulation of large cells about small arterioles in the pulp is slightly more marked in this case than in Case I. In neither case is there the high grade hemosiderosis of the sinus endothelium that Pick describes. With Weigert's iron hematoxylin on frozen sections the large cells stain a pale blue-gray which is much lighter than the dark blue-black of the nuclei. This, too, is clearly a case of Gaucher's disease.

At the time of writing, five and one half years after operation, the patient is feeling unusually well. There is, as yet, no evidence of progression of the disease.

Niemann's Disease

Among the cases reported in the literature as examples of Gaucher's disease, but which on critical examination fail to fulfill the criteria of this disease, are four outlined below which are almost identical. They are neither morphologically nor chemically Gaucher's disease. The credit for the recognition of these cases as forming a distinct disease complex belongs to Pick, although Mandlebaum and Downey [8] decided that two of the cases, those of Knox, Wahl and Schmeisser,[9] should not be regarded as Gaucher's disease. The first of the four cases was described by Niemann [10] in 1914 under the title of "Ein unbekanntes Krankheitsbild." The following abstract of his report is given in some detail.

Irene D. was 17 months old on admission to the clinic. She had had difficulty in digesting her food from the second month on. The abdomen was markedly swollen because of a greatly enlarged spleen. On admission she was very poorly nourished, very apathetic and backward in development. The fontanelle was wide open. There was complete absence of any static function. The skin was flabby and very pale; the face was of a strikingly pale, brownish

color. A slight obliquity of the eyes together with several bluish spots in the skin of the back suggested the mongoloid. Breathing through the nose was markedly hindered and snorting. The abdomen measured 50 cm. in circumference. The spleen reached below the umbilicus; the liver extended to the anterior superior spine. The abdominal veins were distended and there was a low-grade ascites. There was some edema of the legs and the eyelids. The blood had a normal corpuscular and hemoglobin content. The Wassermann reaction was strongly positive. Mercury and potassium iodide were given energetically with negative results. A diarrhea set in and the child at times was slightly febrile. The child died at the age of 18 months after four weeks in the hospital.

Necropsy: There was no evidence of syphilis. The spleen was very large and fairly hard with a somewhat mottled surface. On cut surface there were yellow-white areas as large as linseeds; these were frequently confluent and between them only small strips of normal spleen. When held at a slight distance the cut surface was entirely yellowish white. The liver was likewise greatly enlarged and closely resembled the fatty liver of phosphorous poisoning. The abdominal lymph nodes were slightly swollen, of a peculiar yellow, fat-like color and rather soft in consistency. The kidneys seemed slightly fatty. The cortex of the very large adrenals was markedly yellow.

Microscopic Examination: The spleen showed but slight traces of normal splenic cells. Almost the entire organ consisted of peculiar cells. These were very large, irregular in outline and placed irregularly against each other. The cytoplasm was strikingly pale and stained red with the Pappenheim method as against the basophilia of the splenic leucocytes. There were many vacuoles in the cytoplasm of the cells. The liver was hard to differentiate from spleen. The whole organ was composed of the characteristic cells arranged in indistinct cords. There was very little connective tissue in the liver. The abdominal lymph nodes contained some large cells scattered in clumps, particularly in close relationship to the connective tissue. The cells of the adrenal could be sharply differentiated from the cells occurring in the spleen and liver in spite of the apparent similarity due to the light protoplasm. With Sudan III the cells gave no typical reaction. The color obtained was a darker and duller red than that given by fatty acids. Niemann concluded, however, "dass es sicher woll um Lipoide handeln könnte."

Niemann had never seen slides of Gaucher's disease. He believed the large cells in his case to be Gaucher cells but thought that Gaucher's disease did not occur in people under 20 years of age. He noticed, as Schultze [11] had in his lipemia case, that the sinus endothelium was uninvolved. He believed his cells, because of their close relationship to connective tissue, to be reticulum cells. He thought his case to be chemically different from those of Schultze and Gaucher, however. He suggested the name "large cell metamorphosis of glands" because the process was not limited to the spleen but involved other glandular organs.

Knox, Wahl and Schmeisser [9] have reported similar cases occurring in two sisters who were Jewish. The first child died at the age of 11 months. She had never taken her feedings well and weighed only 8½ lbs. at death. Blood examinations at various times showed a white count varying from 25,400 per c.mm. with 59 per cent polymorphonuclear and 36 per cent small mononuclear leucocytes, to 35,000 with 82 per cent small lymphocytes and 3 per cent polymorpho-

nuclear leucocytes the day before death. The child had a peculiar brownish color of the skin. The course was afebrile.

Necropsy: The liver and spleen were found to be greatly enlarged. Microscopically, large pale cells were found in the alveoli and perivascular connective tissue of the lungs, the blood sinuses of the liver, the pulp of the spleen and lymph nodes, the interlobular connective tissue of the pancreas, in some of the glomerular tufts in the kidneys, the medullae of the adrenals, the stroma and lymph follicles of the mucosa of the intestines and in the pulp of the thymus. Frozen sections showed the cells to be filled with refractile vacuoles soluble in absolute alcohol. These vacuoles were tinged light orange with Sudan III; with Nile blue they were violet-blue or pink. The cells were black by the Weigert-Pal method and many contained doubly refractile granules. "In most of the organs, especially in the liver, spleen and lymph glands, there were all transitions between cells that did not stain at all and those which contained deep orange-staining droplets with scarlet red."

Their second case was a sister of the one described above. The skin had a grayish yellow color. The superficial abdominal veins were dilated. The spleen and liver were both enlarged. There were 3,024,000 red blood cells with 11,240 white blood cells per c.mm., 60 per cent lymphocytes and 34 per cent polymorphonuclear leucocytes. Several months later the white blood count was 22,000 with 31 per cent polymorphonuclear and 67 per cent mononuclear leucocytes. The child was given radium treatment over the spleen after a biopsy on cervical and axillary lymph nodes had given a diagnosis of Gaucher's disease. Several months later the white blood count was 2,360. Examination of the eye grounds showed the cherry spot typical of amaurotic family idiocy. The child died at the age of 15 months. At necropsy the findings were quite similar to those of the preceding case. Large pale cells were noticed in the arteries and veins of the lungs, intrahepatic branches of the portal veins, the splenic arteries and capillaries of the kidneys. The young foam cells were thought closely to resemble the polyblasts of connective tissue.

Siegmund [12] reported a similar case of a girl 9 months old. The child had a large spleen and liver. The large pale cells were found in the spleen, liver, bone marrow, lymph nodes and glomeruli of the kidneys. The splenic sinus endothelium was uninvolved, although many of these large cells were in and between the sinuses. The cells stained very faintly with scarlet red and Nile blue. They were bright yellow after mordanting in potassium bichromate. They also stained with the Ciaccio, Smith-Dietrich, and Weigert's iron hematoxylin methods. Siegmund believed his case to be an early stage of Gaucher's disease.

In the cases of Niemann, of Knox, Wahl and Schmeisser and of Siegmund the patients were girls who died at the ages of 18, 11, 15 and 9 months respectively. These cases were characterized clinically by their appearance in infancy, the failure of the infants to develop, by anemia, leucocytosis, and by a large spleen and liver. In none was there evidence of diabetes or lipemia during life or at necropsy. Anatomically these cases were characterized by the presence of lipoid-containing cells in the pulp of the spleen and lymph nodes, in the thymus, mucosa of the intestine, and medulla of the adrenals, and by the presence of lipoid material in the clasmatocytes of connective tissue, in the Kupffer cells and in large cells lying free within the alveoli of the lungs. The infiltrated cells gave faint reactions with Sudan III and Nile blue but stained deeply with these dyes after mordanting in potassium bichromate and by the method of Lorrain Smith. Occasionally there were large numbers of anisotropic bodies.

Report of Three Cases of Niemann's Disease

We desire to report three similar cases. Two of these were discovered at necropsy and one was recognized during life. From the latter the spleen and a portion of the liver were removed at operation.

Case A. C. K., No. 103427, a Jewish female child 16 months old, was admitted to the service of Dr. Julius Hess, Dec. 5, 1917, with the complaints of stationary weight, diarrhea and underdevelopment. The child did not gain during the first three months. She was tried on various feedings none of which was beneficial. The child was badly constipated. She developed pneumonia. Various types of feedings were administered after this with no success. Shortly before admission a diarrhea began. The stools then contained mucus and small amounts of blood.

Physical examination showed a poorly nourished, anemic girl baby with a red macular rash on the left side of the face and thorax. The fontanelle was still open and depressed. The throat was slightly injected. A few cervical glands were palpable. The lungs were resonant and there were a few râles at the bases. The abdomen was distended and tense. The liver edge was sharp and firm and was palpated four fingers' breadth below the costal margin. The spleen was palpated two fingers below the costal margin; the border was hard and sharp. The umbilicus was somewhat pointed. The skin lacked the normal elasticity and was somewhat bronzed. Roentgenograms of the chest revealed a marked mottling throughout both right and left lungs, which extended well out toward the periphery and was suggestive of tuberculosis. The urine was normal. The Wassermann reaction was negative. The blood examinations showed on Dec. 5, 1917: hemoglobin 90 per cent; red blood cells 4,860,000 and white blood cells 9,100 per c.mm.; neutrophiles 28 per cent; small mononuclears 54 per cent; large mononuclears 10 per cent; transitionals 3 per cent; eosinophiles 3 per cent; basophiles 2 per cent. On December 12th the blood had: red blood cells 4,700,000 and white blood cells 19,600 per c.mm.; neutrophiles 50 per cent; small mononuclears 29 per cent; large mononuclears 15 per cent; transitionals 5 per cent; eosinophiles 2 per cent; basophiles 1 per cent. On December 16th there were red blood cells 4,900,000 and white blood cells 36,000 per c.mm.; neutrophiles 53 per cent; small mononuclears 34 per cent; large mononuclears 8 per cent; transitionals 4 per cent; eosinophiles 1 per cent; hemoglobin 70 per cent. The von Pirquet test was negative.

Necropsy: A necropsy was done by Dr. Perlstein with the following findings.

External Examination: The body is that of a markedly emaciated female infant. The anterior fontanelle is open and measures 2.5 × 2.5 cm. The posterior fontanelle is closed. The pupils are normal. The corneae are cloudy. The very large abdomen is tense and the umbilicus is protuberant. The spleen can be felt to extend down to the crest of the ilium. The liver can be felt 6 cm. below the lower

end of the sternum in the sternal line. There are numerous bluish brown discolorations of the skin, which are present only on the right side of the thorax and abdomen. They are well-defined, round in shape and measure up to 0.4 cm. in diameter. The glands in the axilla and groin can be felt.

Internal Examination: Very little subcutaneous fat is present. When the peritoneum is incised some clear yellowish fluid escapes. The omentum is dotted with numerous small white nodules which measure up to 2 mm. in diameter. They have some similarity to tubercles. Hardly any fat is left in the omentum. The spleen and liver are very large and occupy the greater part of the abdominal cavity. In the dependent portions of the thorax there is also a considerable quantity of fluid.

Heart: The heart is of normal size. The valves are normal. The endocardium is smooth and glistening and the myocardium has the normal color.

Lungs: The lungs are large, dark red in color, congested and edematous. On section a blood-tinged frothy serum escapes. Nowhere can induration be felt. The peribronchial lymph nodes are not enlarged.

Thymus: The thymus weighs 8 gm. It is pale and appears edematous.

Liver: The liver measures 20 × 12 × 6 cm. and weighs 580 gm. Its surface is smooth but slightly uneven. It is pale yellow to salmon-pink in color. On section it is of the same color and fatty to touch. Throughout the organ the central veins stand out as small minute dark red dots in the yellowish pink ground substance. The gall bladder and bile passages are normal.

Spleen: The spleen measures 12.5 × 7.5 × 3.5 cm. and weighs 190 gm. The surface is smooth, the capsule tense and the organ firm. It is pale pink with some irregular areas of a darker red scattered through it, giving it a mottled appearance. Its edges are rounded. On section it is pale pink and scattered through it are some irregular dark red areas which have the color of normal splenic tissue. The splenic veins are of normal appearance.

Kidneys: The left kidney measures 7.5 × 3.5 × 1.5 cm. and weighs 30 gm. The right kidney measures 7 × 4 × 2 cm. and weighs 32 gm. The capsules of both strip without difficulty. On section the kidneys are paler than normal. The anatomic landmarks

are well defined although the tissue is somewhat cloudy. The cortex in its thickest portion measures 0.6 cm.

Adrenals: The right adrenal measures 4 × 2.4 × 1 cm. and weighs 5 to 6 gm. Its cortex is prominent and thicker than normal. The left adrenal measures 3.8 × 3 × 1 cm. and weighs 8 gm. It is of the same appearance as the right adrenal.

Pancreas: The pancreas weighs 18 gm.; it is pale, softer than normal and feels fatty.

Intestines: The intestines are pale. In the lower portion of the ileum a number of enlarged Peyer's patches are seen. They are pinkish red and slightly raised over the surface of the grayish mucosa. They are 2 to 3 cm. long and about 1 cm. wide. A few of these patches are seen farther up in the ileum, but none is seen in the jejunum. The mucosa of the large intestine is studded with enlarged lymph follicles which are especially numerous in the proximal portion and which project from the mucosa as small nodules. They measure up to a few millimeters in diameter.

The *lymph nodes* of the mesentery are markedly increased in size and number. They measure up to 1.5 cm. in diameter and are pale and of a grayish color on section. Several large lymph nodes are present in the meso-appendix. The retro-peritoneal lymph nodes are increased in size.

Microscopic Examination: All sections were made by the writer from tissues fixed in formalin and preserved in 80 per cent alcohol since 1917.

Heart: The heart muscle fibers in places are slightly vacuolated and are suggestively embryonal in character in that the fibrils are grouped around the periphery of the sheath and the centers of the fibers are vacuolated.

Thymus: Most of the organ has been replaced by pale cells, the process being much more marked in the cortex than in the medulla. The comparatively few collections of lymphoid cells are, for the most part, rather compactly arranged around the Hassall's bodies. In some places the pale cells come almost up to the Hassall's bodies (Fig. 3). With Mallory's aniline blue the cells stain a delicate vacuolated blue; some are multinucleated. Thin strips of reticulum separate the pale cells into large groups. The reticulum, however, does not always extend around the individual cells. In the areolar tissue between the lobules, the large pale cells, which here stain with

a little more red than within the lobules, are in closer relation to the reticulum and collagen fibrils.

Lungs: Sections taken through various parts of the lungs show most of the alveoli to be filled with coagulated albuminous material. Polymorphonuclear leucocytes are present in a few of the alveoli. Many of the alveolar spaces are occupied by large foam cells which are frequently multinucleated (Fig. 4). Some of these cells are much larger than the usual dust cells. The capillaries in the alveolar wall not infrequently contain similar cells, which usually are smaller than the large cells in the alveolar spaces and in the branches of the pulmonary artery. In another section there is a small lymphoid area consisting of a few lymphoid cells, typical lymph sinuses and large numbers of pale vacuolated cells apparently identical with those in the mesenteric lymph nodes and the spleen. These cells do not radiate in strands along the contiguous alveolar septa. One septum, however, is quite rich in these cells, and with the aniline blue stain one gets the impression that they may be contained within lymphatics. In the branches of the pulmonary artery there are quite a few of the large cells, sometimes as many as three and four in a single high power field. The large cells are also very frequent in the connective tissue about the arteries and bronchi.

Liver: In the hemalum and eosin preparation the liver cells stain lightly and are interspersed with the pale-staining cells described above. There is a slight increase in the periportal tissue, and not infrequently there are small groups of lymphocytes and polymorphonuclear leucocytes in the periportal areas. With the low power it is very difficult to delineate the cords of liver cells. With the high dry objective, however, the liver cells are finely stippled, pink-staining cells with round, rather darkly staining nuclei. Between the liver cords pale-staining cells lie apparently in the sinusoids. Few sinusoids contain blood and practically no normal Kupffer cells can be recognized. The nuclei of the pale cells vary much in shape; some are round, others are irregular in shape, and still others are markedly elongated. The cytoplasm of these cells is distinctly vacuolar in character (Fig. 5). Occasionally long spindle-shaped nuclei line the spaces containing these large cells. With the aniline blue stain the distinction between the liver cells and pale cells is well brought out. The liver cells stain a coarsely granular, orange-red; the nuclei vary from yellow to orange-brown, while the pale

cells are light blue. The reticulum and collagen are unusually well demonstrated. Each liver cord is bounded by a thin strip of dark blue reticulum. The pale cells with their vacuolated cytoplasm lie, for the most part, between the liver cell cords and the covering reticulum. In the periportal areas there is a marked increase in reticulum. In these areas the pale cells predominate, sometimes almost to the entire exclusion of liver cells in large portions of a high power field. With this stain none of the cells has the longitudinal striation characteristic of Gaucher cells.

Spleen: With hemalum and eosin the organ bears very little resemblance to a normal spleen; it is made up practically entirely of groups of pale cells (Fig. 6). A few strands of thick, deeply red-staining fibrous tissue run through the organ and the capsule is normal in appearance. With some difficulty the sinuses free of foam cells can be picked out. With Mallory's aniline blue stain, however, the sinuses stand out quite prominent with the low power as thin, double-walled, purplish blue cords against the fairly homogeneous bluish gray background composed of closely packed foam cells (Fig. 7). With the higher power the sinus endothelium is low and flat, and an occasional sinus lumen contains a few comparatively small foam cells; sometimes, however, a foam cell as large as those in the pulp is seen. In places, at dilatations of the sinuses, the pulp appears to be pressing into the lumen of the sinus. The sinuses are compressed by the foam cells outside them. The Malpighian bodies consist almost entirely of foam cells. The latter are closely packed about the vessels. In tracing the trabeculae one finds the blue-staining collagen in places suddenly spreading out in finger-like processes into the reticulum fibrils of the pulp. These fibrils are separated by small numbers of foam cells. The foam cells proper are usually round, oval or slightly polygonal in shape and vary in size from 20 to 60 micra. The nuclei are comparatively small; occasionally a cell is multinucleated. The cytoplasm is very finely vacuolated; but in some cells the vacuoles have coalesced, so that the whole cytoplasm may consist of two or three large vacuoles.

Pancreas: The pancreas itself presents nothing abnormal and is surrounded by fetal fat. A few pale cells with various degrees of vacuolization lie in the connective tissue about the organ. This is particularly well marked around a comparatively large vein which runs through the section.

Mesenteric Lymph Node: A preparation stained with Mallory's eosin-methylene blue or hemalum and eosin shows the node to be composed of diffusely distributed large cells with pale, faintly vacuolated cytoplasm and relatively small nuclei (Fig. 8). The cells are confined almost entirely to the pulp. Large pale-staining cells are more numerous in the centers than in the peripheries of the few typical follicles present. The lymph sinuses are not particularly prominent and they do not contain any of the large pale cells. The loose areolar tissue surrounding the lymph nodes contains many of the pale cells which are smaller than those in the lymph node proper. The nucleus is eccentric and small, and apparently all gradations can be traced between small, oval or spindle-shaped cells with slightly elongated nuclei, through cells of the same shape containing finely vacuolated pale-staining cytoplasm, up to the typical pale cell occurring in the pulp of the spleen. The earliest stage of this process involves what are probably young reticulum cells. Section stained with aniline blue shows the reticulum fibrils very nicely. Most of the lymph sinuses are free from large cells and the pulp is the portion apparently most affected. With this stain the cytoplasm of the pale cells is quite prominent, because of a very definite light blue vacuolization. Nothing suggestive of the typical longitudinal striation of the Gaucher cell is seen. In the loose areolar tissue about the node the pale cells are much more prominent with this stain than with hemalum and eosin and the transition stages are even more plainly marked by this method.

Kidney: The tubular epithelium in places is quite thin and finely vacuolated. Some of the glomeruli contain large, pale, finely vacuolated cells in their tufts (Fig. 9). The very small branches of the renal artery contain these cells, which are frequently found in the arterioles up to the Malpighian bodies.

Adrenal: The cells of the cortex are slightly vacuolated. The medulla is greatly thickened with large numbers of foam cells. These are most numerous at the cortico-medullary junction. Many of them, however, lie within capillaries. With Mallory's aniline blue stain the blue foam cells stand out quite sharp against the red of the cortical and medullary cells.

Small Intestine: Scattered through the lower layers of the mucosa are a few large pale-staining cells; these are not prominent and are usually spheroidal. The nucleus contains a very darkly staining

nucleolus and a very indistinct chromatin network. Heidenhain's iron hematoxylin shows the vacuolated appearance of the large cells very beautifully. In some of the cells the vacuoles are quite small and in others they are confluent and form large droplets. The large cells are quite abundant, in one section, in the lymph follicles of the mucosa. There are quite a few of the large cells in the fibrous portion of the submucosa.

With special staining methods for lipoids the pale cells scattered throughout the various organs in this case give practically uniform reactions. With Sudan III the cells take on a yellowish brown color. When these sections are counterstained with hemalum the cytoplasm takes up some of the hemalum so that the appearance is a grayish brown; with Nile blue sulphate the foam cells stain a very delicate lavender. After mordanting in potassium bichromate and staining with Sudan III the large cells contain variously sized granules of highly refractile bright yellow material. In similarly prepared sections stained with Nile blue sulphate the cytoplasm of the large cells is filled with lavender granules. In frozen sections stained with Weigert's iron hematoxylin the cytoplasm of the large cells is composed of dark bluish black granules. In places the cells have taken on so much of the stain that the nuclei are completely obscured. In the lung the large cells also take the Weigert's iron hematoxylin and large black cells are seen in the branches of the pulmonary artery. With this stain, too, the large cells in the medullae of the adrenals stand out unusually prominently. The large cells in this case do not react positively to tests for iron. In frozen sections their cytoplasm is light blue with hemalum and purplish red with alum carmine. The basophilia of the cytoplasm disappears after treatment with absolute alcohol.

Case B. No. 62969, a Jewish female infant 14 months old was admitted to the pediatric service of Dr. I. A. Abt on Feb. 25, 1925, and discharged March 19th.

The infant's development had been rather slow. She held her head up at 4 months. She could not sit alone nor stand at the time of admission. The first tooth had appeared at 9 months. She had been breast fed for 7 months and then various feeding mixtures were tried. Orange juice and cod liver oil had been administered in large amounts. The family history was unimportant.

At the age of 7 months the mother noted that the infant would take no other feeding than milk and also that it made no effort to sit up or move. A physician was consulted who found nothing wrong with the baby. She continued a subnormal development and did not smile or seem as active as she should be. When

the baby was about 8 months old the mother noted an enlargement of the abdomen which a physician said was due to an enlarged liver and spleen. At the age of 12 months the adenoids were removed. The baby had been keeping her mouth open for various lengths of time. She nursed readily but vomited the 6 o'clock feeding occasionally. The bowel movements were normal. She had lost 1 lb. in the past month.

Physical examination revealed a markedly anemic and deformed child who was quiet during the entire examination. The fontanelle was nearly closed. The pupils did not react to light. The conjunctivae were subicteric. The upper lids were swollen and there was a fine nystagmus of both eyes. Breathing was obstructed, and the tonsils were enlarged. The lips were very pale. The cervical lymph nodes were palpable on both sides. The lungs were normal. The lower thoracic spine was kyphotic and the ribs flared out. The abdomen was quite prominent and distended. The liver was palpable as far down as the anterior superior spine. The spleen extended on the left side to the anterior superior spine; the notch was palpable at the level of the umbilicus. No fluid wave was made out in the abdomen. The legs were quite small and there was a bilateral talipes. The left hand had only the distal phalanges of four fingers. The epiphyses of all the bones were enlarged. The knee and ankle reflexes were not obtainable.

Splenic puncture was done on March 3rd. The material obtained was a light, yellowish pink bit of tissue measuring 2 × 2 × 1 mm. It was touched to several cover slips, a few cells were teased in salt solution and the remainder of the tissue dropped into 10 per cent formalin. Examination of the material in salt solution showed many red and white blood cells amongst which were scattered a few very large cells measuring 30 to 40 micra in diameter. They were opaque yellowish gray, and with higher magnification were irregular in outline as though the cytoplasm was composed of many variously sized bubbles. When Sudan III was applied to these cells they became orange-gray in color. The bit of tissue placed in formalin was imbedded in paraffin and serial sections cut. These were mordanted in 1 per cent chromic acid and stained with Mallory's aniline blue. The greater part of the section was made up of very large, vacuolated, faintly blue-staining cells which surrounded the few sinuses in the section. The picture so closely resembled that in the sections of the spleen from Case A that the diagnosis was ventured that the case under observation was very probably quite similar to that one.

Laboratory Findings: Hemolysis was complete with 0.25 per cent NaCl, and partial with 0.4 per cent. The urine was normal. Blood examinations: March 1st: red blood cells 4,200,000 and white blood cells 23,400 per c.mm.; neutrophiles 45 per cent; small mononuclears 37 per cent; large mononuclears 8 per cent; transitionals 7 per cent; myelocytes 3 per cent. March 13th: hemoglobin 45 per cent; red blood cells 3,560,000 per c.mm.; neutrophiles 40 per cent; small mononuclears 42 per cent; large mononuclears 8 per cent; transitionals 7 per cent; myelocytes 3 per cent. The erythrocytes showed a moderate degree of anisocytosis, poikylocytosis and polychromatophilia. No nucleated red blood cells were seen. The von Pirquet test was negative. The bleeding time was 6¼ minutes. Before operation the blood cholesterol by the method of Bloor was 210 mg. and the blood fatty acids by the method of Bloor were 640 mg. per 100 c.c. of serum.

On March 9, 1925, splenectomy was performed.

Gross Examination: A piece of liver excised at the time of operating is yellowish pink and measures 4 × 4 × 3 mm. The spleen measures 13 × 8 × 4 cm. and weighs 268 gm. One small notch is 1.5 cm. deep. One aberrant vessel enters the spleen 3 cm. from the hilum. The spleen exteriorly is a light salmon color. The capsule is rather thin and contains frequent, slightly depressed pin-point-sized areas. On section the spleen is salmon-yellow. Scattered through the pulp are small, slightly translucent, grayish pink areas usually less than 1 mm. in diameter. These usually have a thin layer of red tissue surrounding them. On holding the organ at various angles one sees numerous, very small, glistening particles making up the pulp; these suggest fine flakes of mica in appearance. The pulp consistency is resilient, slightly doughy and poor in connective tissue. The trabeculae are not prominent.

Microscopic Examination: The spleen is practically a duplicate of that of Case A. When stained with hemalum and eosin the great mass of the organ is composed of pale-staining cells with small nuclei and a vacuolated pink cytoplasm. The cells are confined almost entirely to the pulp, usually in the vicinity of small arterioles. The lining of the sinusoids is apparently uninvolved in the process, although there are occasional foam cells in the lumen of the sinuses. The reticulum in the centers of the Malpighian bodies is quite prominent and there are a few foam cells in the germinal centers. Hematopoiesis occurs in some of the sinuses. With Mallory's aniline blue stain the distribution of the foam cells is even more characteristic. They have a distinctly blue, vacuolated cytoplasm. Nothing suggestive of the longitudinal striation of the Gaucher cell is seen.

The liver at first glance shows quite an atypical structure. With hemalum and eosin a slight tendency of the cells to group themselves into two classes can be made out. One group stains lighter than the other with the eosin. With Mallory's aniline blue stain the difference between the two types of cells is striking. One group, apparently hepatic parenchyma, stains reddish brown and is coarsely granular. The other type of cell stains distinctly blue and is practically always in places which are surrounded by fairly thin strands of blue-staining reticulum. These cells are clearly vacuolated. There is an increase in the periportal fibrous tissue and the lobules are smaller than usual.

In frozen sections after formalin fixation stained by the Lorrain

Smith-Dietrich method the pulp is yellow except for the black nuclei. Large irregular and rounded black spots corresponding in size and position to the pale cells are scattered throughout the section. Several of these are seen in the light yellow Malpighian corpuscles. By Fischler's method no soap is present. In sections fixed in Flemming's osmic acid mixture the pale cells are dark because of very many fine black granules in the cytoplasm. With hemalum and Sudan III on frozen sections the large cells are finely granular and purplish brown. With hemalum alone the granulation is dark blue. With Sudan III alone the pale cells are a light though brilliant orange; the foreign substance therefore stains with both Sudan III and hemalum. In frozen sections after extraction with acetone or absolute alcohol the pale cells are very light blue with hemalum but do not take the Sudan III. The blue is not as intense as before extraction. The sections of spleen do not react with microchemical methods for iron. With Nile blue the pale cells are a definite light lavender. Frozen, formalin fixed, section of the spleen treated with Weigert's iron hematoxylin is practically a duplicate of that seen in the spleen of Case A (Fig. 10). The large cells are almost black and stand out in prominent clumps and cords against the light bluish gray pulp. The perivascular arrangement in this section is not as prominent as it is in that of Case A, but the presence of the cells along the trabeculae is just as marked.

After mordanting in potassium bichromate and staining with Sudan III the large cells contain very many highly refractile, bright orange granules. In many of the cells the intensity of the orange varies somewhat. In sections mordanted in potassium bichromate and stained with Nile blue sulphate the large cells contain a finely granular deep lavender material. The remainder of the section has a hardly perceptible blue color.

CASE C. W. K., No. A63717, a Jewish male infant, aged 7 months, was admitted to the pediatric service of Dr. I. A. Abt on Mar. 26, 1925. He died the following day.

The infant had been apparently quite normal in development until three days before admission, when he began to cough. His temperature rose as high as 104.5 and the child became semi-comatose. He lay in bed without moving, with his eyes closed and refused to take any food.

Physical examination on admission to the hospital revealed a well developed and well nourished male infant 7 months of age who was very acutely ill. The cheeks were flushed and hot and the skin was dry. The lips were cracked and dry. Respirations were rapid. There was marked nystagmus. The chest was

normally resonant throughout. A few scattered coarse râles and some areas of roughened breath sounds were detected in the chest. The spleen was enlarged, and was palpable 3 cm. below the costal arch. The liver was also palpable. There was an occasional convulsion with tonic contractions of the legs. The knee jerk was decreased on the right side; the Brudzinski was negative; Kernig's and Babinski's signs were positive on both sides; the upper extremities were spastic. Shortly after admission to the hospital the infant began having convulsions with twitching of the face and right arm. It was given chloral and morphine, which stopped the twitching. By spinal puncture markedly turbid fluid was obtained. On smear examination this showed a few red blood corpuscles and many gram-positive diplococci which were occasionally arranged in chains. The infant died that night in a convulsion. Blood examination on March 26th showed hemoglobin 65 per cent; red blood cells 3,960,000 and white blood cells 29,200 per c.mm.

Necropsy: External Examination: The body is that of a well nourished male infant, which measures 66 cm. in length. There are crusts of an eruption in the scalp above the anterior fontanelle; the latter is almost completely closed. The scalp is quite thin.

Internal Examination: Head: The bones of the skull present no external abnormalities; they are thin and can be cut easily with the saw. The dura, which is separated from the calvarium with some difficulty in the region of the sutures between the bones, is thin and non-adherent to the leptomeninges which, over the anterior portions of the cerebrum, are greenish yellow and are raised above the cerebral cortex for at least 1 cm. by a yellowish green fluid exudate. The vessels of the pia are surrounded by a similar exudate which here is slightly more abundant, so that the course of the vessels is marked out distinctly. Portions of the posterior lobes of the cerebrum are free from the exudate. The meninges at the base of the brain, especially in the interpeduncular space, are covered and thickened by an exudate similar in appearance to that described above. The brain substance proper and the ventricular system are free from involvement in the inflammatory process. The cerebrospinal fluid is slightly cloudy. Both middle ears are filled with yellowish green pus.

The hypophysis is small and covered with a slight amount of greenish fibrin. The longitudinal sinus and all of the sinuses at the base of the skull are incised and appear normal. The cribiform plate is removed and the nasal cavity examined; it is apparently normal.

The subcutaneous fat of the anterior abdominal wall is somewhat tallow-like in appearance and measures 1 cm. in thickness. The abdominal and pleural cavities are free of fluid and adhesions.

The *thymus* is quite small and white. It measures 2.5 × 2 × 0.5 cm.

The *lungs* contain air throughout. They are reddish in appearance. No masses are felt within them. On section the pulmonary tissue is mottled red, gray and pink. There is no evidence of consolidation. The cut surface is rather dry and sticky.

The *heart* lies free in the pericardial cavity, which is free from fluid. The heart is of normal size. The ductus arteriosus is present, but its lumen is obliterated. The musculature of the heart is reddish brown and rather firm. The right auricle is distended with clotted blood. On incising the heart no abnormalities are noted in the valves and myocardium.

The *marrow* of the ribs is red.

In the abdominal cavity it is noted at once that the *spleen* is increased in size. The spleen measures 11 × 5 × 3 cm. and weighs 62 gm. Its surface is salmon-pink in color and slightly irregular in contour. A notch extending 2 cm. into the substance of the organ is seen on the gastric surface. On section the pulp is resilient and rather firm. It is salmon-colored, tinged slightly with brown. The Malpighian bodies stand out as small translucent grayish white points surrounded by a thin zone of red which demarcates them from the lighter colored splenic pulp.

The *adrenals* are small and contain but little lipoid. The *gastro-intestinal tract* appears normal, as does the *pancreas*.

The *liver* is of normal size, light brown and the lobulation fairly distinct. The centers of the lobules have a very obvious light grayish tan color in contrast with the surrounding brown periphery.

The *mesentery* contains a few small lymph nodes. The omentum is normal.

The *kidneys* are of normal size and are light brown in color. The capsule is removed without difficulty. There are no hemorrhages in the cortex. The striations are distinct. The bladder and ureters are normal.

No *lymph nodes* are noted as being increased in size anywhere in the body.

Bacteriologic Examination: The meningeal and otitic exudates contained Type II pneumococci in pure culture.

Microscopic Examination: *Thymus:* There is no subdivision of the organ into cortex and medulla. It consists of lobules of thymic

tissue fairly heavily infiltrated with fibrous tissue and small amounts of adipose tissue. Scattered between the few lymphoid cells are large numbers of foam cells with decidedly pink-staining cytoplasm. The Hassall's bodies are prominent. The foam cells are distributed throughout the section and do not seem to have any particular arrangement other than a diffuse dispersement throughout the pulp, as seen by the hemalum and eosin stain. With Mallory's aniline blue stain the foam cells are seen usually to be very close to reticulum fibrils. Most of the blood vessels are free of these cells. About an occasional small vessel the foam cells are somewhat more numerous than elsewhere. The loose areolar tissue about the thymus contains a few foam cells.

Heart: The fibers are quite small, closely placed, and the striation is rather indistinct. In places the fibers are somewhat embryonal in character. The vessels in the heart muscle contain no foam cells.

Lung: Many of the alveoli are completely filled with large foam cells which are quite similar to those described in the spleen and other organs. The cells are most commonly present in alveoli adjacent to septa and beneath the pleura. Some of the alveoli contain only a few of these foam cells, which are spheroidal or polygonal in shape; those which are spheroidal are usually still attached to the walls of the alveoli. In another section of lung the large cells so completely fill the alveoli as in places to give the impression of a catarrhal pneumonia. The bronchi frequently contain large numbers of foam cells, and many are seen in the branches of the pulmonary artery.

Spleen: In the hemalum and eosin preparation, most of the organ consists of groups of large, pale-staining cells, which are 30 to 50 micra in diameter and contain a pink-staining, finely reticular cytoplasm. The type of nucleus varies. In some of the cells it is somewhat vesicular, and contains one or two small chromatin dots. In others the nuclei are quite compact and deeply stained. The cells are present diffusely throughout the tissue, but they are more numerous immediately beneath the capsule and about the trabeculae than in the remainder of the organ. The Malpighian bodies are small and are frequently surrounded by a pulp rich in red cells. Some of the germinal centers are almost completely replaced by foam cells. The same section stained with Mallory's aniline blue shows the grouping of the foam cells much more markedly than does

the routine hemalum and eosin. The venous sinuses occasionally contain a foam cell but for the most part are free. The foam cells lie in the spaces between the sinusoids and practically always surround a small vessel, as shown by the concentric arrangement of the blue-staining reticulum. The picture is quite similar to that described for certain cases of Gaucher's disease by Pick. The endothelium of the sinuses is flattened. In some areas one can trace a direct continuity between the mass of foam cells in the pulp and the lumen of a sinus. The foam cells within the sinuses of the spleen are usually smaller than those in the pulp. Occasionally one finds a comparatively large area of uninvolved pulp, but even here on close examination one can find, contiguous to loose strands of reticulum, foam cells which with the Mallory stain stand out quite sharp because of their reticulated blue cytoplasm. Study of the spleen offers fairly conclusive morphologic evidence that the foam cells in this organ arise from the cells of the reticulum, particularly about the smaller arteries. The endothelium of the sinuses is apparently uninvolved in the formation of the pale cells.

Liver: With the hemalum and eosin stain the liver has a fairly normal appearance. The cytoplasm of the liver cells stains homogeneously red with eosin and most of the Kupffer cells are inconspicuous; a few, however, are large and their cytoplasm is markedly vacuolated. Some of these cells are three and four times the size of the circumjacent liver cells. With the Mallory stain, however, one can trace an apparent gradation between the Kupffer cells with little cytoplasm through others which contain a few small vacuoles, up to the large cells which are preponderatingly vacuolated and are typical of the foam cells found throughout the other organs.

Duodenum: In the lower layers of the mucosa and submucosa are a few small foam cells embedded in fibrous tissue. Their nuclei are compact and deeply stained.

Mesenteric Lymph Node: Foam cells form fairly large clumps in the medullary portion. The peripheral lymphoid follicles occasionally contain cells with large, finely vacuolated cytoplasm. The latter are smaller than the cells seen in other organs and in the medullary portion of the node. The lymph follicles are small.

Adrenal: The cells of the zona fasiculata are somewhat vacuolated; those of the zona reticularis stain well and abut directly against a thick layer of foam cells in the medulla. The latter stand

out sharply against those of the zona reticularis, in that their axes are perpendicular to those of the cellular elements of the deepest portion of the cortex. Beneath this medullary layer of foam cells is a thin zone of engorged capillaries, which frequently contain foam cells. The original medullary cells are in general quite inconspicuous, but are more prominent about the large blood vessels. The same section stained with Mallory's aniline blue shows a similar picture, except that the foam cells stain blue by this method and stand out with even more sharpness against the medulla and cortex. Thick strands of reticulum frequently run between small groups of the foam cells and their intravascular localization is exceedingly well brought out by this method.

Kidneys: The glomeruli are small. The tubules are somewhat swollen. The vessels and glomerular tufts are free from foam cells. In a branch of the renal artery near the pelvis there are quite a few such cells. Section through the other kidney shows this to resemble the one just described, but no foam cells are found.

The subcutaneous fat is apparently normal. Striped muscle presents no abnormalities and is free from foam cells.

Brain: The meninges are greatly thickened with an exudate consisting almost entirely of polymorphonuclear, a few mononuclear white cells and fibrin. In the meningeal exudate only a few large foam cells are seen. No cells of this type are present in the vessels of the meninges. In one section the cortex is apparently normal, except for marked distention of the small vessels with blood. Another section through the brain shows in some places marked infiltration of the walls of the arteries with polymorphonuclear leucocytes, and in other areas well marked perivascular infiltration with the same cells. There is, however, in these areas no suggestion of the foam cells described in the other organs. The cerebellum shows slight infiltration of polymorphonuclear leucocytes in the meninges but no foam cells. The stroma of the choroid plexus is heavily infiltrated with cells which have a large amount of clear cytoplasm. These are the size of the foam cells present in the other tissues, but the cytoplasm has a peculiar striation rather than vacuolization and these are probably not identical with the foam cells in the rest of the body. The pars glandularis of the hypophysis is covered with a thick layer of purulent exudate similar to that observed in the meninges. The organ is hyperemic and in the center there is an ill-

defined area the size of a high power field which contains some cloudy albuminous fluid, imbedded in which are many mononuclear cells. These are larger than the monocyte of the blood and contain darkly staining, slightly vesicular nuclei and finely reticular cytoplasm. They are highly suggestive of young foam cells. The subcutaneous fibrous tissue is apparently normal.

Marrow: The bone marrow contains an occasional foam cell.

Aorta: The aorta and periaortic areolar tissue are normal.

Spleen: With Weigert's iron hematoxylin the light blue splenic pulp stands out sharply against the dark blue-black, opaque, large cells which are arranged in cords and clumps. The small arteries passing through the pulp are sheathed by a layer, two to three cells thick, of the large cells which also run in thin sheets along the connective tissue trabeculae. The cytoplasm of the deeply stained cells is filled with bluish black granules.

Liver: When stained with the same method the liver cells are diffusely bluish gray and are darker at the peripheries of the lobules than in the centers. Scattered in places throughout the organ are greatly enlarged Kupffer cells which stain almost coal black. With the higher powers they are finely granular. A careful study of the Kupffer cells throughout the organ shows most of them to stain deeply, but it is in the greatly enlarged Kupffer cells, particularly about the periphery of some of the lobules, that the black staining of these cells is unusually prominent.

Lung: The large cells in the alveoli of the lung stain intensely black with Weigert's iron hematoxylin. For the most part they are free in the air spaces, usually in those close to the larger bronchi, about the larger vessels and beneath the pleura. A few of the large, deeply stained cells are present in the capillaries and some of the smaller arteries contain similar cells.

After treatment with potassium bichromate followed by Nile blue, the liver cells are sprinkled with dark blue droplets and in places a few rods. The Kupffer cells occasionally are slightly enlarged and contain fairly large granules of light purple-staining material. The pale cells of the spleen are distinctly purple with this method.

The liver stained with Sudan III after mordanting with potassium bichromate shows many fine, yellow granules scattered throughout the liver cells. The Kupffer cells not infrequently contain small amounts of bright orange staining material.

Discussion

In the three cases just described one apparently is dealing with the same process, which is characterized anatomically by the appearance of large foam cells in the various organs examined. The spleens of all three cases are filled to practically the same degree by large pale-staining cells, which stain positively with the special methods for the more complex lipoids, reactions which indicate that part of the stored material is probably a phosphatid. In the sections of all the spleens only an occasional foam cell is found free in the lumina of the sinuses. The endothelium of the sinuses apparently is uninvolved in the process. The Malpighian bodies are involved quite late in the disease. In the livers of Cases A and B there is practically complete change of the Kupffer cells into the large lipoid containing ones. In Case C, however, in which the child died of pneumococcus meningitis before the process had reached complete development, only some of the Kupffer cells are greatly swollen with lipoid, but practically all of them with Mallory's aniline blue show at least beginning evidences of vacuolization and with Weigert's iron hematoxylin evidences of increased lipoid content. In the lymph nodes the lipoid-containing cells lie outside the sinuses. The clasmatocytes or tissue histiocytes, particularly in the loose fibrous tissue about the various organs, are vacuolated and stain positively for the complex lipoids. The thymus, which was examined in Cases A and C, shows a high lipoid content in the vacuoles of the reticulum cells. The alveoli of the lungs in Cases A and C are filled, sometimes almost completely, with the large lipoid-containing cells. In the lungs of both of these cases the same large cells are present in the loose connective tissue about the vessels, beneath the pleura and in the lumina of the arteries. In the kidney of Case A, large cells are found not only in the capillaries and smaller arteries, but also within the glomerular tufts where some of the cells are of unusual size. There are a few large cells in the bone marrow of Case C and in the smaller vessels in the pancreas.

Our three cases anatomically and microchemically are quite similar to the case described by Niemann and simulate even more closely those described in detail by Knox, Wahl and Schmeisser, and by Siegmund. In spite of the fact that these men believed their cases to be typical examples or precursors of Gaucher's disease, there is prac-

tically no evidence for this, as we have shown above not only from the cases of Gaucher's disease reported here but also from those in the literature. The cells in Gaucher's disease are not so uniformly round, are much more frequently fusiform or elongated and do not give the lipoid-staining reactions of the large cells in Niemann's disease. The substance present in the large cells of Gaucher's disease is apparently insoluble in absolute alcohol, while it is taken out with ease by this solvent in the case of Niemann's disease. Moreover, the cells in Gaucher's disease have a homogeneous, rather refractile cytoplasm which with certain stains presents a peculiar longitudinal striation. In Niemann's type, however, the cells either fresh or when treated with the lipoid stains present a very definitely granular appearance. After treatment with the proper solvents they become highly vacuolated. Perhaps the most striking point of difference lies in the distribution of the cells involved in the two cases. Gaucher's disease with marked regularity involves the pulp and sinuses of the spleen, the periportal connective tissue in the liver and the reticulum cells of the lymph nodes and bone marrow. True Gaucher cells have never been seen outside these situations except in the case of Risel, who found a small collection of such cells in a lymphoid follicle in the thyroid. In Niemann's disease, on the other hand, the large cells with equal regularity are present in the pulp of the spleen, in the thymus, and in the mucosa and submucosa of the intestines; they lie free in the alveoli of the lungs and they are found in the blood vessels of the lungs, kidneys and pancreas; the Kupffer cells of the liver and the tissue histiocytes in general become transformed into the large cells. The types of cells involved are apparently in both conditions members of the reticulo-endothelial system, but the two types of disease show strikingly the differences in reaction of various members of this group.

In experimental hypercholesterolemia Anitschkow [13] was able to produce in the spleen, adrenal, liver, wall of the aorta and in the bone marrow, large cells which contained a vacuolated cytoplasm when stained with the usual technic but which gave markedly positive reactions with Sudan III, Nile blue and the Lorrain Smith-Dietrich method. The fat was not seen in the lymphoid follicles and was present along the adventitia of the arterioles. In one rabbit he noticed that cholesterol and trypan blue were taken up by the same cells. In cases of diabetes with lipemia, in the human, Lutz [14] and

Schultze [11] showed the presence of large lipoid-containing cells in the spleen. Williams and Dresbach [15] in a similar case found the large cells in the liver, lymph nodes and adrenals, and Smith [16] in another case found the lipoid material almost entirely in the spleen but also to a small extent in the liver and lymph nodes. Fahr and Stamm [17] have reported a case in a young girl suffering with a mild atypical diabetes in which the large cells were widely distributed throughout the body. In the lipemia cases the lipoid reactions were very much more difficult to obtain than in the experimental cholesterol-feeding work of Anitschkow. It seems probable that some, at least, of the lipoid material in the lipemia cases is of the nature of the complex lipoids, such as the phosphatids, which are so plentifully present in the cases of Niemann's disease.

A point which we have much difficulty in understanding is the marked involvement of the thymus. Embryogenetically the reticulum cells of the thymus are epithelial in origin. Vital staining experiments have given contradictory evidence as to the ability of these cells to take up vital dyes; the majority opinion is that the thymus does not store them. Aschoff,[18] reviewing the question recently, remarked that the thymus probably can store, but that the point requires reinvestigation. Lubarsch [19] reports the frequent finding of iron and fatty substances in the reticulum cells of the thymus of poorly nourished infants in the first year of life. He therefore suggests that thymic reticulum cells should be included, along with certain other cells, in the reticulo-endothelial system. In any event, it is highly interesting that the thymus had stored large amounts of the lipoid material in five of the six cases examined post mortem. It is perhaps still more striking that the thymus should have taken up this material so much earlier than the usually highly phagocytic Kupffer cells did in our Case C. In this case the lungs were richly supplied with the pale cells and one wonders whether the absence of them from the liver may possibly have been due to their having been swept into the lungs. The finding of the large lipoid-containing cells in branches of the pulmonary artery is more than suggestive of the fact that the foam cells lying free in the alveoli are brought to the lung from the blood stream and removed in the lung. It is possible that some of the cells, too, may have developed from the histiocytes present in the connective tissue of the interlobular septa, about the walls of the bronchi and larger vessels,

because with appropriate stains large numbers of lipoid-containing cells are seen in these situations. Apparently here they are not within lymphatics.

In our Case B of Niemann's disease the condition was diagnosed *in vivo* before operation. A sample of blood taken before operation showed the cholesterol and fatty acid content of the blood to be approximately normal. We have no evidence, from this single determination, therefore, that the disease is due to an upset of some sort in the lipoid metabolism other than the evidence of intracellular lipoid accumulations in widespread locations throughout the body.

The cases described above illustrate most beautifully the selective action of similar cells belonging supposedly to the same general group, for there are certain marked differences between the reactions of these cells in Gaucher's disease, Niemann's disease, diabetic lipemia, experimental cholesterolemia and their response to vital stains. The Gaucher substance is probably cerebrin or an allied substance, while the stored material in Niemann's disease according to Siegmund is probably a phosphatid. Gaucher cells, moreover, take up varying amounts of iron and this material is apparently absent in the cells of Niemann's disease. In any event, the cases of Niemann's disease here reported appear to us to be exquisite examples of a pathologic physiologic storage, in contradistinction to the storage of foreign material such as some of the colloidal dyes. We cannot answer the question as to whether the appearance of the large cells is due to an infiltrative or a degenerative process. We have as yet no evidence of lipemia.

Our Case B improved markedly for a period of two months as a result of splenectomy; it then gradually resumed its preoperative condition.* It is hoped that in the future similar cases will receive more careful study from the point of view of the lipoids of the blood and the effect of feeding various diets. The question as to the value of splenectomy requires further time for final answer.

In addition to the anatomic differences described above, Gaucher's disease clinically stands in sharp contrast to Niemann's disease. Gaucher's disease may start early in life but it extends over a long period of years, while Niemann's disease affects infants, none of

*Since the manuscript was sent to press, information has been received that this child died in the latter part of September, 1925, in another city; so far as we know necropsy was not done.

whom has lived as long as two years. There is a leucopenia in Gaucher's disease and a fairly high leucocytosis in Niemann's disease. Various congenital abnormalities have been associated with the few cases of Niemann's disease on record. There was a mongoloid appearance, with difficulty in respiration in two of the seven cases, and amaurotic family idiocy in a third case. Niemann's disease appears to be a deep-reaching disturbance in metabolism, in which the infants do not thrive and there is a piling up of lipoid material in phagocytic cells throughout the body. Gaucher's disease on the other hand is apparently not accompanied by this profound metabolic disturbance and the individuals so affected may live as long as thirty-nine years, as in Schlagenhaufer's [20] case. Five of the seven cases of Niemann's disease occurred in Jews. Six of these seven were girls and only one was a boy. Both of our cases of Gaucher's disease were Jews and although many of the reported instances of this condition were in Jews, the incidence of this disease is not as highly restricted to this race as it seems to be in Niemann's disease.

As a result of our study we feel that a sharp distinction should be made, in agreement with the work of Pick, between Gaucher's disease and the condition first described by Niemann. Niemann suggested for this disease the name of "large cell metamorphosis of glands." We do not feel that this is a particularly appropriate name in that it connotes nothing of the lipoid material stored in these cells; nor do we think the designation of Pick as "lipoid splenohepatomegaly" especially fitting, because in the first place the process is spread through practically the entire body, and in the second place in one of our cases which was fairly well advanced, the liver was just showing beginning involvement. For the want of a better name we would suggest "lipoid-histiocytosis" as tending to convey the idea of a process involving the storing of lipoid material by the histiocytes throughout the body.

Summary

Two cases are reported, a boy aged 6 years and a woman aged 42 years, in whom there was a history of long-standing increase in the size of the spleen, leucopenia, mild secondary anemia, and epistaxis in one case and uterine bleeding in the other.

Microscopically there are large pale cells which contain iron and give no typical reactions with the usual lipoid stains in the spleen, liver and lymph nodes. These large cells have a marked longitudinal striation with Mallory's aniline blue connective tissue method. These two cases seem to be typical of Gaucher's disease.

Three cases of the condition first described by Niemann are reported in infants aged 18, 14 and 7 months. The history in the first two of these was of long continued disturbance in feeding and a failure of the infants to develop. The third patient died of meningitis at a comparatively early period in the disease. One case was recognized *in vivo* and improved a good deal for two months after splenectomy, but then began to fail again.

Anatomically these cases have a widespread infiltration of lipoid material in the reticulum cells of the spleen, lymph nodes and thymus, Kupffer cells, clasmatocytes of connective tissue, and the large cells in the alveoli and perivascular tissues of the lung. The large foam cells are also present in the arteries of the lungs, pancreas and kidneys, and in the branches of the portal vein. In sharp contrast to the Gaucher cells, these cells stain positively for the complex lipoids, they do not contain iron and are markedly vacuolated after treatment with absolute alcohol. The absence of the large cells from blood smears can probably be explained on the basis of their being filtered out by the capillaries, especially those of the lungs.

Chemical analysis of these spleens is now under way and will be reported elsewhere.

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DESCRIPTION OF PLATES XCVI–C

FIG. 1. Spleen of Case I. Gaucher's Disease. Hemalum and eosin stain. X 240.

FIG. 2. Liver of Case I, showing marked longitudinal fibrillation of the cytoplasm of the Gaucher cells. Mallory's aniline blue connective tissue stain. X 560.

FIG. 3. Thymus of Case A, of Niemann's disease. Foam cells are very close to the Hassall's body. A capillary to the right of the latter is partially filled with a foam cell. Weigert's iron hematoxylin stain. X 560.

FIG. 4. Lung of Case A. This shows several alveoli filled with large pale cells. In the alveolus in the upper left hand corner several of the cells are multinucleated. Hemalum and eosin stain. X 240.

FIG. 5. Liver of Case A. The dark liver cells stand out sharp against the pale cells in the sinusoids. A large foam cell near the center of the field contains two nuclei. Weigert's iron hematoxylin stain. X 560.

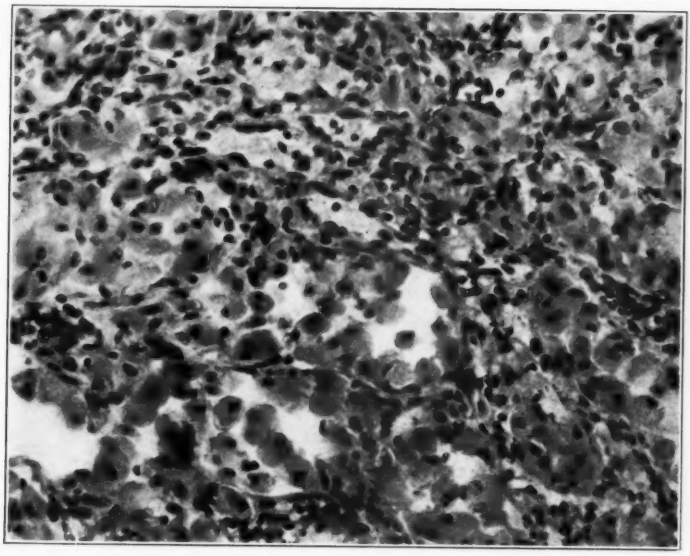
FIG. 6. Spleen of Case A showing the large foam cells in the pulp and the sinuses free of these cells. Delafield's hematoxylin and eosin azur II. X 140.

FIG. 7. Spleen of Case A showing foam cells in pulp and a Malpighian body in the center of the section. The sinusoids stand out clearly and are free of large cells. Mallory's aniline blue stain. X 240.

FIG. 8. Mesenteric lymph node of Case A showing the large vacuolated cells. Heidenhain's iron hematoxylin stain. X 700.

FIG. 9. Kidney of Case A. The right border of the glomerular tuft contains a large foam cell. Another pale cell in the center of the glomerulus is slightly out of focus. Weigert's iron hematoxylin stain. X 560.

FIG. 10. Spleen of Case B of Niemann's disease. The large cells stain an intense black. A few of them are seen about the arteriole in the center of the field. The sinuses are free of large cells. Frozen section after formalin fixation. Weigert's iron hematoxylin stain. X 60.



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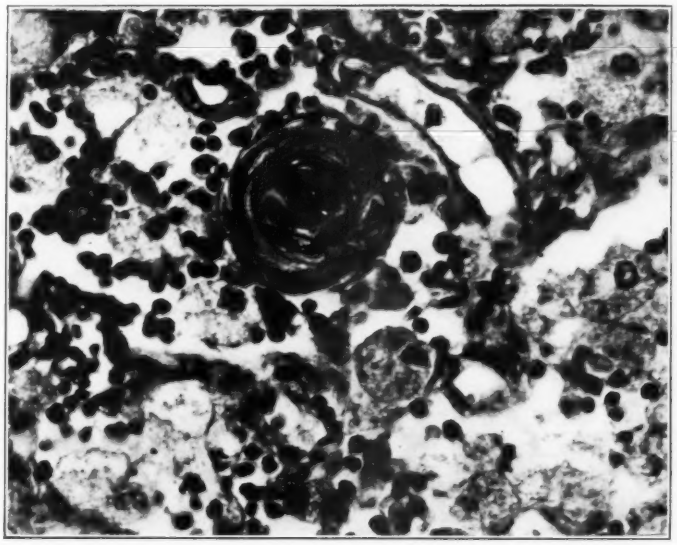

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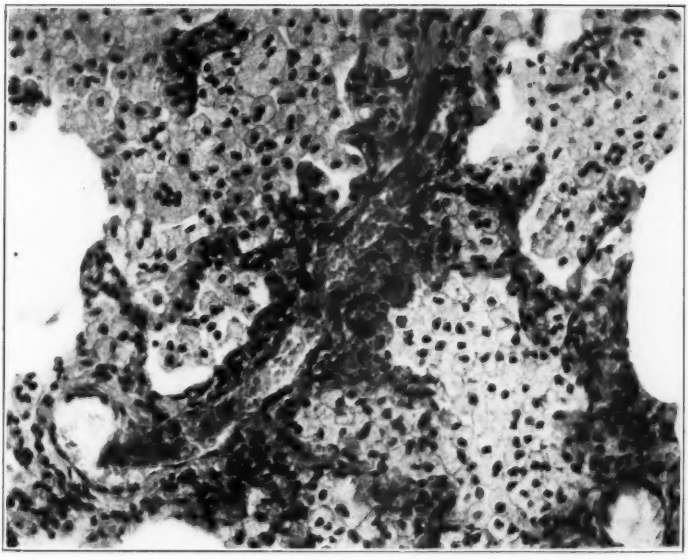

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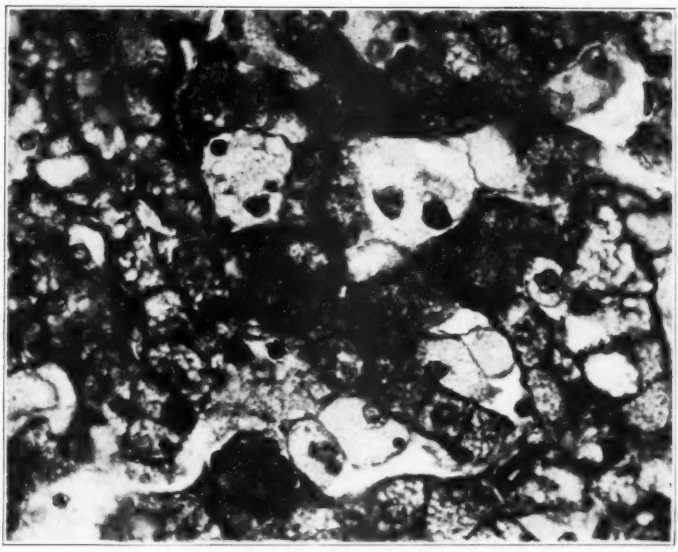

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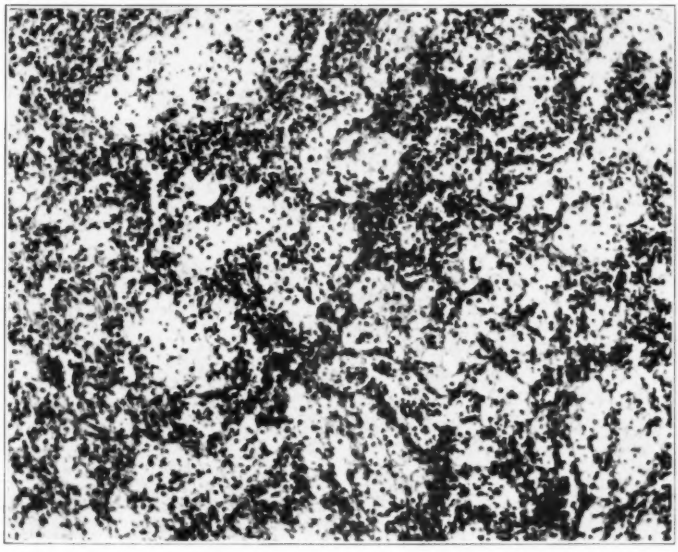

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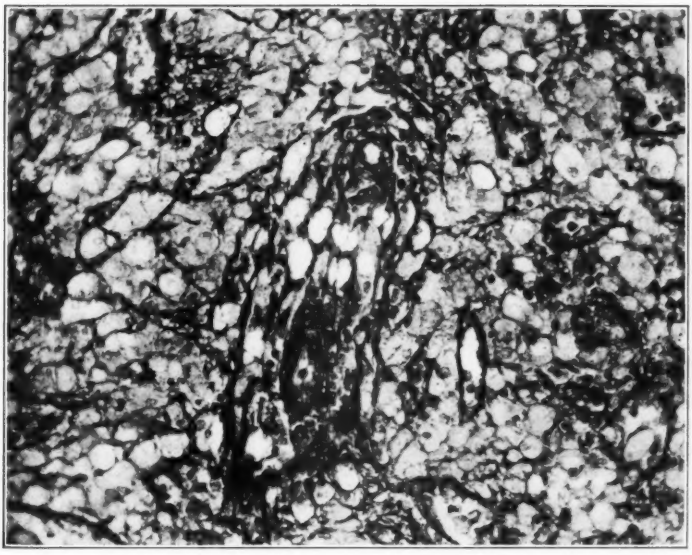

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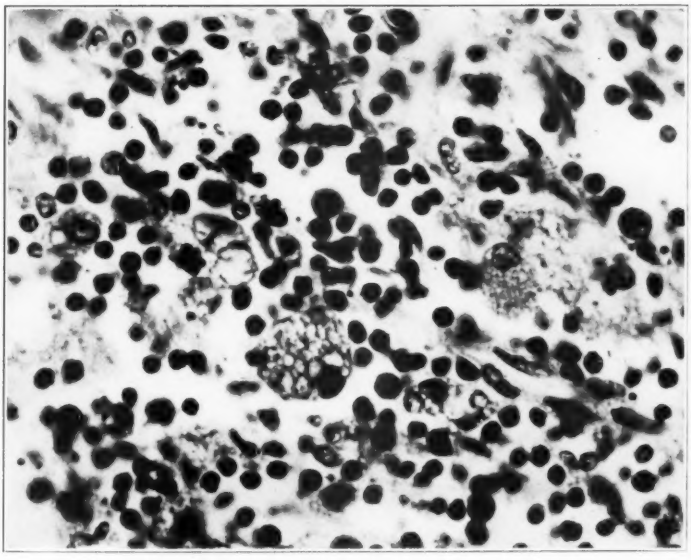

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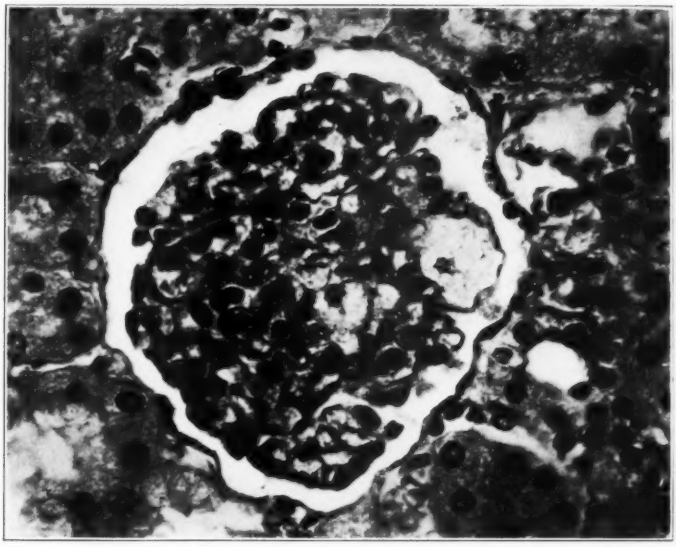

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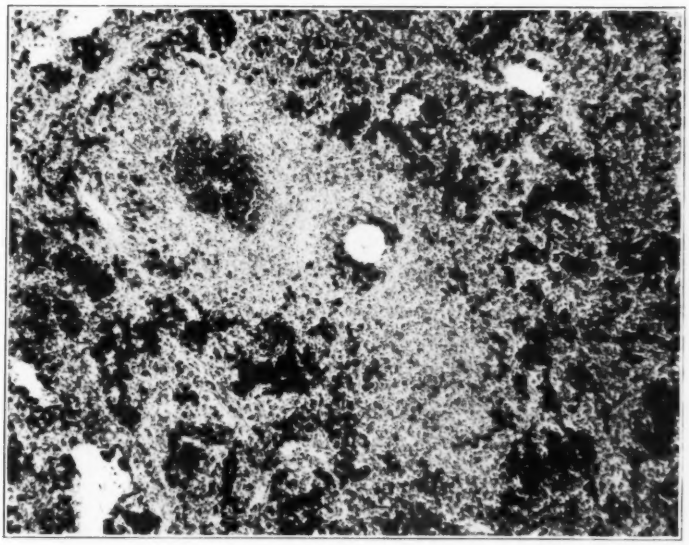

10

EPENDYMOMAS OF THE LATERAL AND FOURTH VENTRICLES OF THE BRAIN *

EDWIN F. HIRSCH AND ARTHUR R. ELLIOTT

(From the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago, Illinois)

Differences are now recognized between tumors arising from the ependyma and those originating from the choroid plexus. This distinction, however, has not been made in many of the reports given in the older literature, although some are excellent and represent good work. Of these older reports some do not mention the important cell characteristics which differentiate between tumors of choroid plexus and those of ependymal origin, while others deny any possibility of distinguishing between cells arising from these different places. This confusion makes almost impossible an accurate summary of the ependymomas now on record. Then, too, for example, the tumor of the brain and cord originally described by Spiller [1] as a glio-sarcoma was later regarded as an ependymoma of the fourth ventricle. There are reports which suggest that the tumor probably is an ependymoma described under another name.

In 1914 Natonek [2] arranged in two groups the tumors of ependymal and choroid plexus origin recorded in the literature:

(1) Tumors of the choroid plexus epithelium
  (a) normal structure
  (b) modified structure

(2) Tumors of the ventricle ependyma
  (a) normal position
  (b) misplaced

This classification in the first group is based on histologic structure but in the second on topography only. The choroid plexus tumors of normal structure are the benign papillomas and those of modified structure are carcinomas of which Natonek thinks there are three, namely: (1) Kaufmann's [3] adenocarcinoma of the lateral ventricle which is described as resembling a malignant bowel polyp, (2) Roki-

* Received for publication August 9, 1925.

tansky's [4] colloid carcinoma of the choroid plexus and (3) Wunscheim's [5] tumor of the fourth ventricle. As characteristics of the ependymal epithelial tumors Natonek emphasizes their multiplicity (local metastases) and their ability to infiltrate. He believes that the one reported by Saxer [6] is the only non-malignant tumor of this kind. All the other ependymal tumors according to him are termed carcinomas and he refers specifically to those reported by Spät,[7] Kolpin,[8] Hart,[9] Cornil,[10] and Bielschowsky and Unger.[11]

Not all the tumors as originally discussed by the authors mentioned come into such a grouping. Kaufmann, for example, states that the tumor he describes had its origin in the ependyma of the lateral ventricle.

Ribbert's [12] paper in 1910 on neuroepithelium in gliomas of the brain and spinal cord mentions similar tumors reported by Stroebe, Saxer, Stolpe, Chiari, Henneberg, and Muthmann and Sauerbeck, [13] and he describes a tumor of the fourth verticle which he says is like the description given by Muthmann and Sauerbeck. This one reported by them, another by Spiller [1] and three by Mallory [14] are considered by Bailey [15] as the only five authentic ependymomas recorded in the literature. He adds six more.

According to Bailey's statements, ependymal cells of the embryo are ciliated, and at the base of each cilium is a small granule known as blepharoplasten. This may be a single granule, a pair of granules one above the other, a minute rod or a dumb-bell-shaped form. For a short period during embryonic life blepharoplasten granules exist in the choroid cells of the lateral ventricles. They are transient in most ependymal cells, but persist in the adult in the cells of the aqueduct of Sylvius, of the rhomboid fossa and of the central canal of the spinal cord. Blepharoplasten granules, according to Bailey, were observed in the ependymal cells by Marchi and were described by Von Engelmann. Weigert subsequently described them in his monograph on the neuroglia to which Mallory refers when he suggests that the presence of these granules is important in establishing the ependymal origin of certain tumors of the brain. Such granules may be demonstrated by any neuroglia stain but Mallory recommends phosphotungstic acid hematoxylin. Bailey states that neuroglia fibrils are formed normally from the ependyma even in postfetal life, and that possibly this mode of growth has a large rôle in certain pathologic conditions.

From these statements, the finding of blepharoplasten granules in cells and of glia fibrils is important in recognizing tumors of ependymal origin. Such a distinguishing feature has not been considered in the description of many of these brain tumors.

Bailey's conception of ependymomas is that they grow slowly and are relatively benign. They should not be removed, he believes, when demonstrated at operation. He reports a patient in excellent health more than ten years after one of these tumors had been demonstrated by operation but left undisturbed. This benign course, however, is not true in every instance, and probably there need be considered two varieties, the one benign, the other malignant with infiltrative qualities, metastases and rapid growth. Just where to make the distinction may sometimes be difficult.

Mallory has described these tumors as gliomas of ependymal origin, while Ribbert speaks of them as gliomas containing neuroepithelium. These designations specify the presence of glia tissue along with the epithelium. Spiller and Bailey use ependymoma as a descriptive name which seems sufficiently simple and accurate when amplified by terms designating benign or malignant qualities.

Histologically the ependymomas are essentially papillary tumors. The individual papilla consists of a central stalk, chiefly a thin-walled blood vessel and very little fibrous tissue covered by several layers of polygonal cells with granular cytoplasm. Where the tumor growth assumes malignant qualities there is a dense palisade of cells several layers deep arranged radially and at right angles to the vessel lumen. In addition, duct-like structures are present in variable numbers. Between the cell masses are delicate or coarse glia fibrils, the number of which varies considerably. Blepharoplasten granules are present in the cytoplasm of the cells. Many of the tumors reported are primary in the fourth ventricle of the brain.

These comments are made as introductory to a report of two ependymomas, one of the left lateral ventricle, the other of the fourth ventricle. The latter ependymoma resembles both grossly and microscopically the tumors of the fourth ventricle reported by Bailey.

G. A., a male 16 years of age, was in good health according to statements by relatives until September, 1923, when he injured the lumbar and sacral region of his back in falling from a tree. An examination by a physician at this time demonstrated no significant injury, but the boy did not return to school,

although he was up and about most of the time. In March, 1924, stiffness of the legs was noted, chiefly difficulty in obtaining full extension. After a month without improvement, tuberculosis of the spine was diagnosed. For a year an afternoon temperature of about 99.6 F. was observed. About ten days before admission to the hospital his physical condition grew worse with loss of appetite and morning attacks of nausea and vomiting. On January 10th, these attacks became so severe that no liquids or solids could be kept in the stomach. His mental state changed in that he was surly, his memory failed and he was disoriented. Spasticity of the legs increased. For nine months there had been blurred vision. The boy was admitted to St. Luke's Hospital, Chicago, Jan. 11, 1925, to the orthopedic service of Dr. E. W. Ryerson and later to the medical service of Dr. A. R. Elliott. The nausea and vomiting continued, emaciation became marked, mental deterioration increased and death occurred March 8th, without a satisfactory clinical diagnosis having been made.

The essential changes demonstrated by necropsy of the trunk, head and neck are confined to the brain in which there is a huge tumor of the left cerebral hemisphere. The description of the tumor follows.

The formalin hardened brain on March 19, 1925, weighs 1616 gm. The right cerebral hemisphere is 17.5 cm. long and 7 cm. wide and the left is 18.5 cm. long and 8 cm. wide. The cerebral convolutions are markedly flattened, especially of the left in its posterior two-thirds. On the medial surface of this left lobe beginning 3 cm. in front of the posterior pole, extending forward 4.5 cm. and vertically 5.5 cm. to the superior surface is a friable gray and white mass which here has completely replaced the brain tissue. Close to the midline on the superior surface and continuous with it is an irregular nodule bulging from the cortex and about 2 cm. in diameter. Slightly in front of this and 5 cm. from the midline is another mass 3.5 x 4 cm. The dura is densely adherent to these places and the vessels covering them are markedly dilated with blood. The leptomeninges otherwise are moderately hyperemic, lie closely in the sulci of the brain and are semitransparent. Immediately behind the optic chiasma and encroaching upon it is a gelatinous gray mass, cone-shaped with its base corresponding to the tip of the infundibulum. This tissue is 2 cm. in diameter at its base and is about 2 cm. high. The aqueduct of Sylvius is patent and pushed slightly to the right of the midline. There are no changes in the lining or plexus of the fourth ventricle. The third ventricle is unchanged except that it has been pushed to the right side of the midline by the tumor mass in the left half of the cerebrum. The right lateral ven-

tricle is markedly distended, the maximum lateral dimension is about 3.2 cm. and the vertical 5.4 cm. Of the left lateral ventricle there is present only the anterior 5 cm. of the cornu anterior and the anterior 1.5 cm. of the cornu posterior. From a coronal section at a level corresponding to the anterior end of the third ventricle and extending backward practically to the posterior tip of the left cerebral hemisphere, a length of about 12 cm. and about 7 cm. in both of its other dimensions, there is a huge tumor (see Figs. 1 and 2) which has entirely replaced the brain substance. In the same regions it has destroyed the cortex. The tumor is soft, easily torn, and yellow and white mottled with old and recent hemorrhages. It contains irregular cyst-like regions, the largest about 2 cm. in diameter, filled with a gelatinous white substance. Portions of the yellow tissue are necrotic. The tumor substance contains many blood vessels of which the largest has a lumen about 4 mm. in diameter and a wall thick with hyaline fibrous tissue. The tumor tissue has replaced the lateral ventricle except the portions mentioned. The choroid plexus of the right lateral ventricle is unchanged, but of the left only the anterior portion remains and it lies on the surface of granular tumor tissue which extends almost to the opening into the third ventricle. The lining of the left lateral ventricle in front is studded with many small masses of similar tissue. One of these on the septum pellucidum is about 15 mm. in its surface dimensions and protrudes 5 mm. In the right lateral ventricle are scattered others from 5 to 8 mm. in diameter. A probe passed through the opening of the lateral ventricle into the third ventricle continues into the tissue mass in the region of the infundibulum. In the coronal section through the right cerebral hemisphere in front of the corpora quadrigeminae there is a gray tumor mass exposed in the temporal lobe immediately beneath the floor of the genu posterior of the ventricle (see Fig. 1). It is about 1.5 cm. in diameter and extends through the brain tissue to the pia.

Thin sections of the tumor tissue prepared from paraffin blocks, stained with hematoxylin and eosin and with phosphotungstic acid hematoxylin, contain tumor tissue with a papillary structure (see Fig. 3). In the center of each papilla is a thin-walled blood vessel on which are four to six layers of polygonal cells, surrounded by a palisade about equally wide of cells more densely arranged and at right angles to the central vessel. Glia fibrils and blepharoplasten

granules (see Fig. 4) are present in the cells stained with phosphotungstic acid hematoxylin. By careful search a few tubules (see Fig. 5) lined with a single layer of cuboidal or low columnar epithelium were found. The papillary structure is present also in the smaller tumor masses of the ventricles, right cerebral hemisphere and infundibulum.

For the second ependymal tumor reported we are indebted to the courtesy of Dr. Dean Lewis. It occurred in a white man 34 years of age, who complained of headache, nausea, vomiting, disturbance of vision and irritability and symptoms, beginning about fifteen months before death, attributed to a brain tumor, possibly of the hypophysis. Three weeks before death a right temporal decompression was done by Dr. Lewis. At the operation a markedly increased intracranial pressure was observed and following the operation brain tissue herniated through the decompression opening of the skull. Death occurred suddenly with severe difficulty in respiration. A postmortem examination of the head only was permitted. Excepting trauma of the cranium, pericranial tissues and brain resulting from the operation, the changes of particular note are confined to the fourth ventricle and the adjacent tissues.

The brain without the dura on July 20, 1925, weighs 1514 gm. The right cerebral hemisphere has the following dimensions: length 17.5 cm., width 7 cm. and thickness 7.5 cm. The respective dimensions of the left are: 18 cm., 8 cm. and 8 cm. The leptomeninges are gray and slightly opaque, the convolutions flattened and the sulci correspondingly narrow. On the right cerebral hemisphere corresponding to the upper end of the fissure of Rolando there is an irregular torn mass of brain substance 4 x 5 cm. in which there are recent hemorrhages occupying about one-fourth of the tissue. This mass projects irregularly from the brain cortex about 2.5 cm. In segments of the brain made in the usual way by cutting in parallel coronal planes about 1.5 cm. apart, there is a slight widening of the left lateral ventricle and the septum pellicidum is pushed to the right. The third ventricle is widely distended by a blood clot which is continuous behind and through the aqueduct of Sylvius into the fourth ventricle. Wedged between the dorsum of the spinal cord and the inferior surface of both lobes of the cerebellum and pushing these structures apart is a smooth gray-white tumor mass 4.5 cm. long, about 3.5 cm. wide and 3.5 cm. thick (see Fig. 6) arising from the

velum posterior. The anterior end of this mass is continuous with the blood clot filling the fourth ventricle and about 2.5 to 3 cm. in its greatest dimensions. On section the tumor tissue is gray-white, friable and mottled with many pin-point and slightly larger hemorrhages. On its dorsal surface immediately below the lobes of the cerebellum the thin fibrous capsule covering this mass is densely adherent to the dura and in removing the brain from the cranium the tissue is torn over an area 2 x 1.5 cm. Adjoining this place there is an erosion of the bone forming the posterior part of the foramen magnum.

Sections stained with hematoxylin and eosin contain cells polygonal in outline in the less dense regions but compressed to a spindle shape in the compact places. There are many thin-walled capillaries (see Fig. 7). The cytoplasm is faintly granular and abundant, the nuclei are vesicular and usually have a single round mass of chromatin close within the nuclear membrane. In sections stained with phosphotungstic acid hematoxylin there are blepharoplasten granules in the cell cytoplasm, but only a few glia fibrils. Most of the cells are relatively immature but in some the cytoplasm is continued into thread-like prolongations in one or more places.

Summary and Conclusions

Two ependymomas of the brain are reported. One, primary in the lateral ventricle, has the infiltrative qualities of a malignant tumor, while the other, in the fourth ventricle, is chiefly an expansile growth pushing aside the adjacent tissues. This difference in growth suggests that certain ependymomas are benign while others are malignant, and that growth characteristics should be recognized in the general class of tumors arising from the ependyma.

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DESCRIPTION OF PLATES CI–CIII

FIG. 1. Photograph illustrating the tumor size in the left cerebral hemisphere at the level of the corpora quadrigeminae, and the tumor metastasis in the right temporal lobe.

FIG. 2. Photograph illustrating the tumor size and appearance in a coronal plane through the middle of the left occipital lobe.

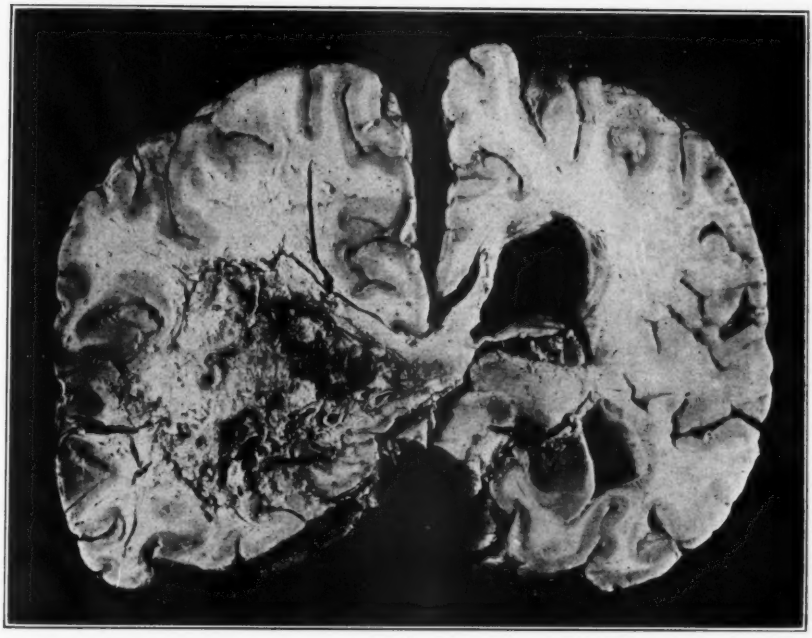
FIG. 3. Photomicrograph to illustrate the palisade arrangement and papillary structure. X 100.

FIG. 4. Photomicrograph illustrating glia fibrils and blepharoplasten granules. X 1200.

FIG. 5. Photomicrograph of the ducts contained in the ependymoma of the lateral ventricle. X 200.

FIG. 6. Photograph of the ependymoma of the fourth ventricle.

FIG. 7. Photomicrograph illustrating the tissue structure of the fourth ventricle ependymoma. X 140.



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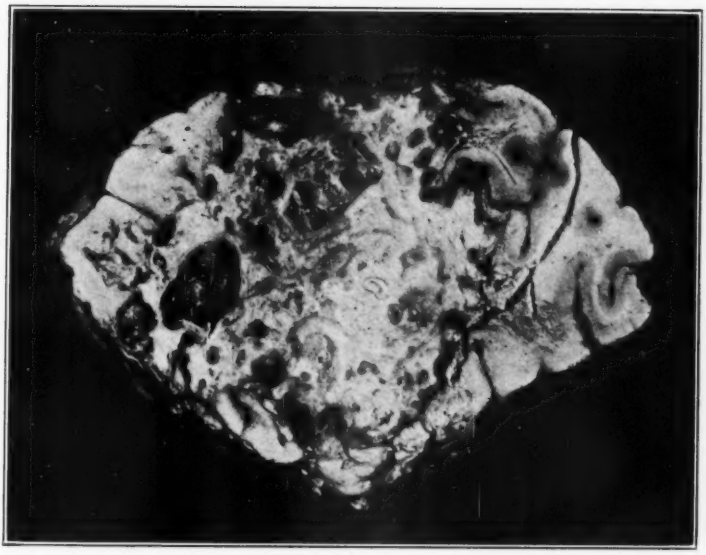

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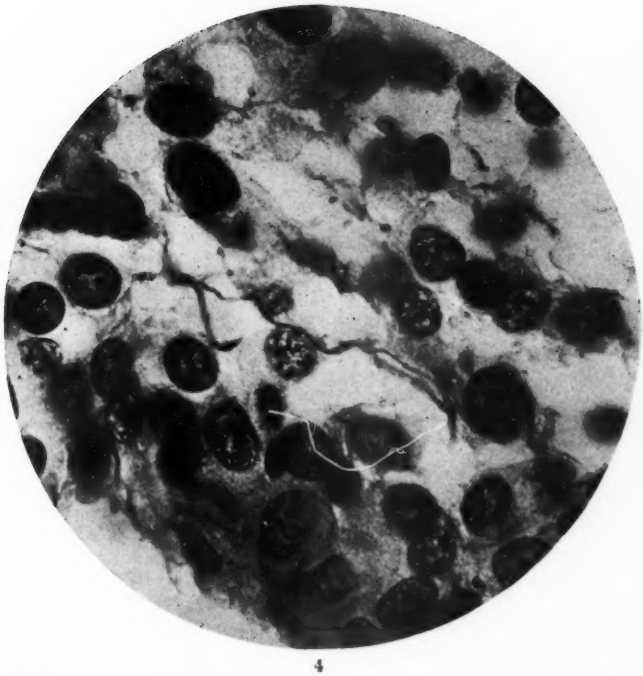

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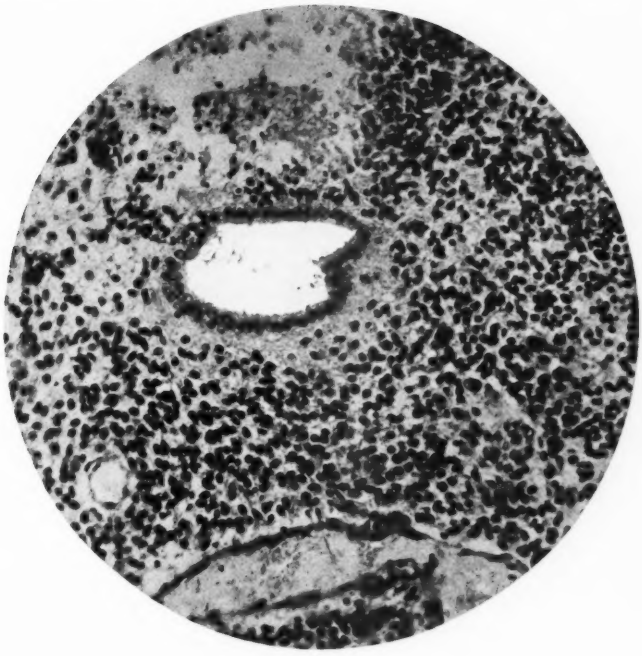

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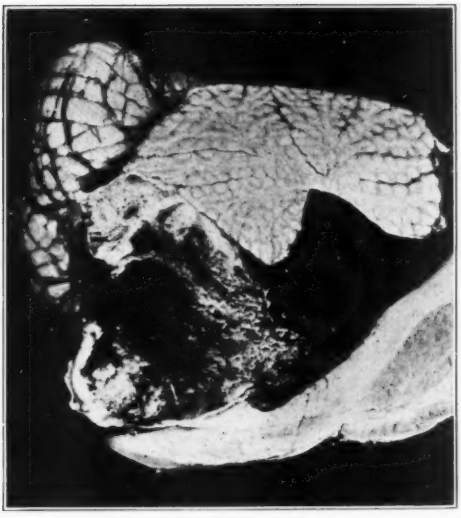

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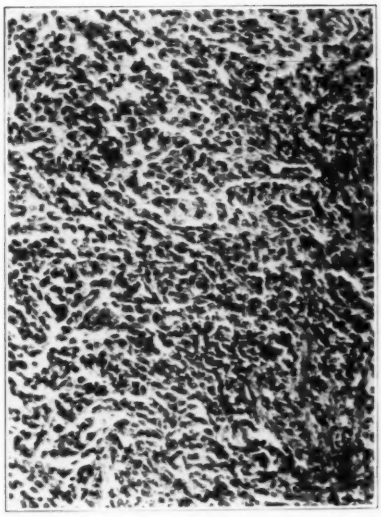

7

Hirsch and Elliott

Ependymomas

RESEARCHES ON VACCINE VIRUS *IN VITRO* WITH SPECIAL REFERENCES TO ITS AFFINITY FOR NERVE TISSUE *

NORIMASA HIRANO

(From the Nagasaki Medical College, Nagasaki, Japan, and the Department of Comparative Pathology, Harvard Medical School, Boston, Massachusetts)

It is well known that when a piece of fresh tissue is put at the bottom of a culture tube, chemical reduction of the culture medium takes place and at the same time several substances dialyze out of the tissue. However, the effect of tissue on micro-organisms under cultivation is still not understood.

The present study was undertaken with two objects in view, first to prove the effect of the tissue on vaccine virus in culture medium; and second to determine whether or not vaccine virus migrates along the tissue *in vitro*.

EXPERIMENTAL METHODS AND DATA

I. COMPARISON OF THE AFFINITY OF VACCINE VIRUS FOR THE KIDNEY AND THE SPINAL CORD

The cornea, skin and testicle of rabbits are suitable places for the growth of vaccine virus *in vivo*, of which the testicle seems to be the most favorable. This has been already proved by the experiments of Noguchi [1] and Henseval.[2] Recently Marie [3] reported that when the virus is inoculated into the brain of a rabbit, it causes the death of the animal after four or five days. Also Levaditi and Nicolau [4] showed that if vaccine virus which had been passed several times through rabbits by intratesticular inoculation is injected into the brain of a rabbit, it causes a fatal infection and they have succeeded in making passages from brain to brain in series. I have found that when a strain of vaccine virus which has been passed through rabbits by intratesticular inoculation without increasing its virulence, is injected into the brain of a rabbit, its virulence increases very quickly. In view of the above facts, there seems to be no doubt that the central nervous system is an especially suitable place for the propagation of vaccine virus. On the contrary, according to Ohta-

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wara [5] and Levaditi and his co-workers, the affinity of the virus for the kidney is said to be either completely lacking or else very slight *in vivo*.

Therefore, I have carried out the following experiments in order to obtain some knowledge as to the affinity of vaccine virus *in vitro* for these tissues, namely, kidney, spinal cord and testicle.

The culture medium employed throughout the experiments on the affinity of virus for tissue was 3 per cent glucose broth containing 1 per cent glycerin with a pH of 7.0 to 7.2.

In every instance the material employed for inoculation into culture medium was the testicular strains of vaccine virus which had been passed several times through rabbits. The stock emulsion of the virus was prepared by grinding up aseptically removed vaccinated testicle with 50 per cent glycerin solution containing 0.5 per cent carbolic acid in the proportion of 1.0 gm. of the tissue to 2.5 or 5.0 c.c. of the solution. After grinding, this emulsion was aspirated with a sterile pipette through sterile gauze and put into a sterile test tube. This test tube was allowed to stand in an icebox for a few days, thus permitting the coarse particles to settle. For the purpose of inoculation the supernatant glycerin was pipetted off and varying dilutions, 1: 2 or 1: 5 in physiologic salt solution, were prepared.

The method employed consisted in inoculating medium from two series of test tubes containing 10 c.c. of the culture medium mentioned above and a piece of fresh sterile tissue (either spinal cord or kidney) at the bottom. Into each tube 0.1 c.c. of the diluted vaccine virus was placed. After inoculation, the tubes were covered with a layer of sterile paraffin oil and placed in an incubator at 37 C. Every twenty-four hours during incubation the test tubes of this series were taken out of the incubator one at a time, and if the culture medium was free of contaminating bacteria, it was transferred into another sterile tube with a capillary pipette. The tissue at the bottom of each tube was washed several times with sterile salt solution and weighed to the milligram. An emulsion of the washed tissue was made by grinding with sterile salt solution in the proportion of 0.1 gm. of the tissue to 1.0 c.c. of salt solution, *i.e.*, 1: 10 dilution of the tissue. For the purpose of inoculation into a rabbit, dilutions of the culture medium and the emulsion of tissue were prepared with sterile salt solution as follows: culture medium, 1: 1, 1: 10 and 1:100; tissue, 1: 10 (original emulsion), 1: 100 and 1: 1000.

The presence of the virus in the various dilutions of culture medium and tissue was tested according to Groth's [6] method of intradermal inoculation, *i.e.*, 0.1 c.c. of each inoculum was injected intradermally into a normal rabbit previously shaved over the sides of the abdomen. Altogether twelve inoculations were made at one time at least an inch apart in a single animal. The rabbits were white healthy adults weighing usually from 1.5 to 2.0 kg. The emulsion of spinal cord and the spinal cord medium were injected on the left side and the emulsion of kidney and the kidney medium on the right side. Although an effort was always made to prevent any of the inoculum escaping into the subcutaneous tissues, it is likely that in some instances this happened to some extent. After inoculation, the skin was observed daily for signs of reaction until inflammatory processes had nearly disappeared.

The results of the experiments are shown in Table I. This table shows clearly that vaccine virus has an affinity for the spinal cord of rabbits but almost no affinity for the kidney. Twenty-four hours after inoculation, the virus was found to be present in both the

TABLE I

Affinity of Nervous Tissue for Vaccine Virus

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
117	24 hours	Emulsion of spinal cord	1:10	+	+	++	++	++	R
			1:100	+	+	++	++	++	R
			1:1000	−	+	++	++	++	R
		Fluid medium	1:1	+	+	+++	+++	+++	R
			1:10	+	+	+++	+++	+++	R
			1:100	?	+	++	++	++	R
		Emulsion of kidney	1:10	?	+	++	++	++	R
			1:100	−	+	++	++	++	R
			1:1000	−	−	+	+	++	R
		Fluid medium	1:1	?	+	+++	+++	+++	R
			1:10	?	+	+++	+++	+++	R
			1:100	−	+	++	++	++	R

+ = visible lesion. The number of plus marks represents the size of the lesion, +++ being the maximum.

− = absence of a visible lesion.

R = inflammatory process retrogressing.

TABLE I (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
118	2 days	Emulsion of spinal cord	1:10	+	++	++	+++	+++	R
			1:100	+	++	++	++	++	R
			1:1000	−	+	+	+	+	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	++	+++	R
		Emulsion of kidney	1:10	−	−	−	+	+	R
			1:100	−	−	−	+	+	R
			1:1000	−	−	−	−	−	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	+	+	R
119	3 days	Emulsion of spinal cord	1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
			1:1000	+	+	+	+	R	
		Fluid medium	1:1	+	+++	+++	+++	R	
			1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
		Emulsion of kidney	1:10	−	−	−	−	−	
			1:100	−	−	−	−	−	
			1:1000	−	−	−	−	−	
		Fluid medium	1:1	+	++	++	+++	R	
			1:10	+	+	+	+	R	
			1:100	−	+	+	+	R	
120	4 days	Emulsion of spinal cord	1:10	+	+	++	++	++	++
			1:100	+	+	+	++	++	++
			1:1000	−	?	+	+	+	+
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	−	?	+	++	++	++
			1:100	−	?	+	++	++	++
		Emulsion of kidney	1:10	−	−	−	?	?	+
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	+	+	+	++	++	++
			1:100	−	?	+	++	++	++

spinal cord and the kidney tissue, each giving similar skin reactions; at the end of forty-eight hours' incubation in the test tube the virus in the spinal cord caused marked inflammatory processes in the skin, and four days later was found to be still active, whereas the incubated kidney showed a marked decrease in virus and an earlier disappearance.

The papules produced by the virus incubated in spinal cord were different from those caused by the virus in kidney or by the fluid portion of either medium. The papules during the active stages of the infection were redder and more prominent, the infiltration more intense and profound. Later they were sometimes covered with a crust which often remained for weeks.

The culture medium containing the spinal cord produced a more active reaction on the skin than that containing the kidney although the difference was by no means marked.

Repetitions of this experiment have in all cases given similar results.

The intradermal injection of an emulsion of a normal spinal cord caused no inflammatory reaction in the skin of rabbits.

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Vaccine virus of the lot previously used was employed in this experiment. It showed, however, a definite decrease of virulence in consequence of long preservation. Thus the inoculation of 0.1 c.c. of 1:100 dilution into the skin of a rabbit produced a definite though not intense inflammatory reaction.

The methods and culture medium employed to determine the effect of nervous tissue on virus of low virulence are similar to those previously used. The results of the experiment are summarized in Table II. It is quite evident that the skin reacted to the 1:10 tissue emulsion, while the medium failed to produce a visible lesion, although the medium of two days' incubation produced a papule. In this experiment, the vaccine virus incubated with the spinal cord for twenty-four hours produced a papule earlier than when incubated a longer time; its inflammatory processes, however, were not intense. On the contrary, the spinal cord which was incubated with the virus two or three days caused a papule later, but its infiltration was more intense and extensive.

TABLE II

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				5	6	7	8	9	10
104	24 hours	Emulsion of spinal cord	1:10	?	+	+	+	R	
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	–	–
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–
105	2 days	Emulsion of spinal cord	1:10	–	–	?	+	+++	+++
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	++	+++
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–
106	3 days	Emulsion of spinal cord	1:10	–	–	–	–	+	+++
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	–	–
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–

For legend see Table I.

The results indicate that the virus survives longer in the nervous tissue than in the medium, and it would appear that the virus diminishes in amount after incubation but increases in virulence.

II. Comparison of the Affinity of Vaccine Virus for the Testicle and the Spinal Cord

The incubation was carried out at 37 C. and at room temperature (about 21 C.). In the case of the incubation at room temperature I gave my attention to obtaining evidence as to whether the tissue, especially the spinal cord at the bottom of the culture tube, had any effect on the virus suspended in the medium, simultaneously with my efforts to compare the affinity of the virus for the testicle and spinal cord.

Table III

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus
(Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
30	24 hours	Emulsion of spinal cord	1: 10	+	+	++	++	R	
			1: 100	−	+	+	++	R	
			1: 500	−	−	−	+	R	
		Fluid medium	1: 1	+	++	+++	+++	R	
			1: 10	?	+	+++	+++	R	
			1: 50	−	−	++	++	R	
		Emulsion of testicle	1: 10	−	+	++	++	R	
			1: 100	−	−	?	+	R	
			1: 500	−	−	−	+	R	
		Fluid medium	1: 1	+	++	+++	+++	R	
			1: 10	+	+	+++	+++	R	
			1: 50	−	−	++	++	R	
31	2 days	Emulsion of spinal cord	1: 10	−	−	+	+	++	+++
			1: 100	−	−	−	+	+	++
			1: 500	−	−	−	−	−	−
		Fluid medium	1: 1	−	−	−	+	++	+++
			1: 10	−	−	−	+	++	+++
			1: 50	−	−	−	−	+	++
		Emulsion of testicle	1: 10	−	−	−	−	?	++
			1: 100	−	−	−	−	−	−
			1: 500	−	−	−	−	−	−
		Fluid medium	1: 1	−	−	+	+	++	+++
			1: 10	−	−	−	+	++	+++
			1: 50	−	−	−	−	+	+
32	3 days	Emulsion of spinal cord	1: 10	+	++	++	R		
			1: 100	+	+	+	R		
			1: 500	−	−	−	−	−	−
		Fluid medium	1: 1	−	−	−	−	−	−
			1: 10	−	−	−	−	−	−
			1: 50	−	−	−	−	−	−
		Emulsion of testicle	1: 10	−	−	−	−	?	−
			1: 100	−	−	−	−	?	−
			1: 500	−	−	−	−	−	−
		Fluid medium	1: 1	−	−	−	−	−	−
			1: 10	−	−	−	−	−	−
			1: 50	−	−	−	−	−	−

For legend see Table I.

The strain of vaccine virus employed in the experiments was inoculated once into the brain of a rabbit and afterwards passed several times through rabbits by intratesticular inoculation. Otherwise it was prepared in the same way as in the previous experiments.

The culture medium and the methods are also similar to those of the previous experiments with the exception that testicular tissue was used in the place of the kidney. In some cases the tissue emulsions were inoculated also into the testicle of a living rabbit. The dilutions of each inoculum were prepared with sterile salt solution as follows: culture medium, 1: 1, 1: 10 and 1: 50; tissue, 1: 10, 1: 100 and 1: 500.

The results of the experiments are shown in Tables III to VI.

According to Table III, the spinal cord incubated with the virus twenty-four hours at 37 C. produced visible lesions on the skin earlier than the testicle produced them. In addition to this, the spinal cord diluted 1: 100 caused a papule on the skin even after three days' incubation, while the testicle similarly incubated produced no visible lesion even when diluted only 1: 10. The activity of both mediums whether containing a piece of spinal cord or testicle was the same. Three days after incubation the virus disappeared from the medium. In other words, as soon as the virus inoculated into the medium containing testicular tissue died out in the medium after incubation, it also disappeared in the tissue. On the contrary, when the virus which was inoculated into the medium containing the spinal cord died out in the medium, it still remained active in the spinal cord even to the following day.

Similar tests for the activity of virus by inoculation into the testicle *in vivo* showed results comparable with those obtained by intracutaneous inoculation as summarized in Table IV, namely, the testicle incubated three days with the virus caused only a very small induration in the testicle while the spinal cord emulsion still produced an intense inflammatory process.

The experiments in which tissues were incubated at room temperature (21 C.), as will be seen in Tables V and VI, gave further evidence of the retention of the virus in the spinal cord. It was noted that the spinal cord incubated with the virus even for twenty-one days was very active and produced a more intense reaction on the skin than when incubated for sixteen days. In other words, the virus in the spinal cord remained very active notwithstanding the

TABLE IV

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle					
				4	5	6	7	8	9
31	2 days	Emulsion of spinal cord	0.2 c.c. (1:10)	–	Slight induration appeared	Induration increased	Induration and swelling	Increasing fullness and firmness	
		Emulsion of testicle	0.2 c.c. (1:10)	–	Slight induration appeared	Induration increased	Induration and swelling	Induration and swelling slightly increased	
32	3 days	Emulsion of spinal cord	0.2 c.c. (1:10)	Slight induration appeared	Induration increased	Marked induration	Fullness and firmness increased	Induration somewhat less than on the previous day	Similar to the previous day
		Emulsion of testicle	0.2 c.c. (1:10)	–	–	–	Indurated spot appeared	Similar to the previous day	Induration disappearing

long incubation. The virus in the testicular tissue, however, became so weak after twenty-one days' incubation that it produced only a slight inflammatory process on the skin and in the testicle, even with an emulsion diluted 1: 10.

When the spinal cord which was incubated with the virus was inoculated into the testicle of rabbits, as will be seen in Table VI, there took place at the end of two or three days, swelling and induration of the organ, which increased in size and density for five or six days.

TABLE V

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
38	5 days	Emulsion of spinal cord	1: 10	+	++	++	++	R
			1: 100	+	++	++	++	R
			1: 500	−	+	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	+	+	++	R	
			1: 50	−	+	+	R	
		Emulsion of testicle	1: 10	+	+	++	++	R
			1: 100	−	+	++	++	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	−	+	++	R	
			1: 50	−	−	+	R	
39	7 days	Emulsion of spinal cord	1: 10	+	++	+++	+++	R
			1: 100	?	++	++	++	R
			1: 500	?	+	+	+	R
		Fluid medium	1: 1	+	+++	+++	R	
			1: 10	+	+	+	R	
			1: 50	?	+	+	R	
		Emulsion of testicle	1: 10	?	++	+++	+++	R
			1: 100	?	?	?	+	R
			1: 500	?	−	−	?	−
		Fluid medium	1: 1	?	++	+++	+++	R
			1: 10	?	+	+	+	R
			1: 50	?	+	+	+	R

For legend see Table I.

TABLE V (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
40	10 days	Emulsion of spinal cord	1: 10	++	++	++	++	R
			1: 100	−	++	++	++	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	++	+++	+++	+++	R
			1: 10	++	++	+++	R	
			1: 50	+	+	++	R	
		Emulsion of testicle	1: 10	+	++	++	R	
			1: 100	−	?	+	R	
			1: 500	−	−	+	R	
		Fluid medium	1: 1	++	+++	+++	R	
			1: 10	+	++	++	R	
			1: 50	−	+	+	R	
41	16 days	Emulsion of spinal cord	1: 10	−	+	+	+	R
			1: 100	−	−	+	+	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	−	−	−	+	R
			1: 10	−	−	−	−	−
			1: 50	−	−	−	−	−
		Emulsion of testicle	1: 10	−	−	+	+	R
			1: 100	−	−	?	+	R
			1: 500	−	−	?	?	R
		Fluid medium	1: 1	−	−	−	+	R
			1: 10	−	−	−	−	−
			1: 50	−	−	−	−	−
43	21 days	Emulsion of spinal cord	1: 10	+	+	++	++	R
			1: 100	−	+	+	++	R
			1: 500	−	?	+	+	R
		Fluid medium	1: 1	+	+	+++	+++	R
			1: 10	−	+	++	++	R
			1: 50	−	+	+	+	R
		Emulsion of testicle	1: 10	?	−	+	+	R
			1: 100	−	−	?	+	R
			1: 500	−	−	−	−	−
		Fluid medium	1: 1	−	+	+	++	R
			1: 10	−	?	+	++	R
			1: 50	−	-	+	+	R

At this time the color of the testicles was purplish red, spotted here and there with irregular yellowish areas of variable dimensions. In sections many polymorphonuclear leucocytes are seen to invade the interstitial tissues together with a serous exudate; the nuclei of cells within the infiltrated areas become karyorrhectic and some tubules are destroyed. The testicular cells are hydropic and fill up the tubular lumen. The virus in the testicle *in vitro*, however, gradually became attenuated after incubation. In consequence of this attenuation in every instance, the virus gave no intense reaction in the testicle with the exception of that of three days' incubation. The virus after incubating three days in medium containing testicular tissue or spinal cord gave almost the same reactions in the testicle up to six days after the inoculation. At the end of seven days, however, definite differences were noted, namely, the testicle inoculated with the testicular emulsion decreased in size, while that inoculated with spinal cord emulsion still showed an intense reaction. At the end of twelve days the former testicle was normal in size, pale and soft and few small grayish foci were recognized. The sections showed only a small infiltrated area. The latter testicle, however, was covered with a grayish fibrous mass adhering to the tunica vaginalis which was still edematous. The sections showed areas of necrotic tissue and extensive infiltration.

The results obtained in these experiments show that the nervous tissue seems to be endowed with more marked affinity for vaccine virus than the testicle. No marked difference was noted in the effect of the tissue of the spinal cord and that of the testicle on the virus suspended in the medium.

III. Effect of the Spinal Cord on Activity of Vaccine Virus

In view of the results obtained in the foregoing experiments it seems proper to raise the question whether the nervous tissue contains any substances which increase the activity of vaccine virus. To determine this question the following experiments were carried out.

A piece of fresh spinal cord and a piece of the spinal cord previously incubated in the medium (glycerin dextrose broth) for two or three days at 37 C. were separately ground with sterile physiologic salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. These tissue emulsions were mixed with the same amounts

TABLE VI

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle				
				3	4	5	6	7
37 *1	3 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Similar to the last
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Marked reduction in size
38	5 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Induration increased accompanied by edema of the scrotum	Similar to the last	Marked swelling and edema of the scrotum
		Emulsion of testicle	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Similar to the last	Similar to the last	Induration not increased
40 *2	10 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	No increase	No increase		
43 *3	21 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration	Marked induration	Marked swelling and edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	No sign	Slight induration	Similar to the last		

*1 Twelve days after inoculation both testicles were removed. *2 and *3 Five days after inoculation both testicles were removed.
All these testicles were fixed in Zenker's fluid and sections cut in paraffin.

of a highly virulent virus (original mixture diluted 1: 100); these mixtures were further diluted 1: 10 with sterile salt solution. Then 0.1 c.c. of each mixture was inoculated into the skin of a normal rabbit. As control, the virus and the emulsion of spinal cord without vaccine virus similarly diluted with sterile physiologic salt solution, were inoculated.

The results of these experiments are shown in Table VII.

TABLE VII

Effect of the Spinal Cord on Vaccine Virus

Rabbit No.	Spinal cord and control	Inoculum	Dilution of inoculum	Size of dose	Reaction at various days after inoculation into skin				
					3	4	5	6	7
62	Fresh	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	+	+	+	+	R
			1: 10	0.1 c.c.	−	+	+	+	R
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	R
			1: 10	0.1 c.c.	−	−	+	+	R
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−
63	2 days incubated	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	+	+	+	R	
			1: 10	0.1 c.c.	+	+	+	R	
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	+	+	R	
			1: 10	0.1 c.c.	−	+	+	R	
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−
64	3 days incubated	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	++
			1: 10	0.1 c.c.	−	−	−	−	+
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	+
			1: 10	0.1 c.c.	−	−	−	−	?
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−

For legend see Table I.

The results show that the virus inoculated with the emulsion of spinal cord caused stronger inflammatory processes in the skin of a normal rabbit than the virus without spinal cord. In view of this fact, there seems no doubt that the spinal cord of a normal rabbit has a suitable substance for promoting the activity of vaccine virus.

IV. Is Vaccine Virus Capable of Migrating along the Spinal Cord *in Vitro*?

To determine this question the following experiments were carried out. A piece of the spinal cord about 6 cm. long was placed straight in a Petri dish. A mixture of 15 per cent agar (one part) and glycerin dextrose broth (one part) was poured into the dish until the piece of

Table VIII

The Question of Migration of Vaccine Virus along Spinal Cord in Vitro

Rabbit No.	Period of incubation	Inoculum	Method of inoculation	Result
54	3 days	1	Intradermal	+++
		2	"	–
		3	"	?
58*	3 days	2	Intratesticular	?
		3	Intradermal	?
59	4 days	1	Intradermal	+++
		2	"	–
		3	"	–

1 = emulsion of tissue of inoculated end.
2 = emulsion of tissue of uninoculated end.
3 = agar surrounding uninoculated end.
* Two weeks after first inoculation revaccination was made into the skin but no response was obtained.

spinal cord was totally covered. After the poured mixture hardened, one end of the piece of spinal cord was exposed by removing a little agar, and at this end a small drop of vaccine virus was inoculated. The dish was placed at room temperature (21 C.). After incubation for different periods of time 0.1 cm. was cut off each end of the spinal cord. These end-parts of tissue were separately emulsified by grinding with sterile salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. A normal animal was inoculated with 0.2 to 0.3 c.c. of the emulsions and also with the agar surrounding the uninoculated end of the cord.

The results of these experiments, as summarized in Table VIII, show that the tissue emulsions of the uninoculated end failed to produce a visible lesion, while in two cases agar surrounding the tissue caused a doubtful reaction on the skin. In view of these facts it is evident that vaccine virus is incapable of migrating along the spinal cord *in vitro*, although there is a possibility that it may be transmitted through a space between the spinal cord and agar (capillary attraction?).

Discussion

According to the embryonic origin of tissues the spinal cord is an invaginated segment of the ectoderm, so that it can be inferred that the central nervous system of the rabbit affords as favorable a site for the multiplication of the virus as the skin. But when the virus is injected into the blood stream, the brain is either deprived or fairly free of vaccine virus. In this connection Levaditi [4] has announced that it is probable that the vascular endothelium (or the entire choroid plexus) prevents the passing of vaccine virus from the blood stream into the nervous system. On the other hand, he produced evidence that the virus grows abundantly in the brain when normal saline or broth is injected into the brain of an intravenously infected animal, because the irritation due to the intracerebral injection of saline or broth causes an aseptic meningitis and breaks down this resistance, thus offering to the virus an excellent culture medium, the brain itself.

My experiments *in vitro* show that the central nervous system of the rabbit is endowed with more marked affinity for the vaccine virus than the testicle. Furthermore it seems as if the virulence of the virus increases in the nervous tissue to some extent. Therefore it is probable that of all tissues the central nervous system contains the most favorable substances and soil for propagation of the virus although the virus does not multiply in the nervous tissue removed from the body.

Steinhardt and his co-workers [7, 8] applied the method of cultivating corneal tissues *in vitro* to the study of vaccine virus and stated that there was slight multiplication of the virus. Recently Parker [9] announced that he had succeeded in carrying vaccine virus through many generations in artificially cultivated testicular tissues. If the virus is really able to grow in tissue cultures, it would be expected

that the central nervous system, being endowed with a specific affinity for the virus, should be the favorable tissue for such a purpose. Experiments to decide this point are now under way.

Summary

1. Vaccine virus has an affinity for the central nervous system but almost no affinity for the kidney *in vitro*, the latter indeed having a deleterious effect on the virus.

2. Vaccine virus has also an affinity for testicular tissue but this is less marked than its affinity for nervous tissue.

3. Vaccine virus survives in nervous tissue immersed in fluid medium after dying out in the latter.

4. Vaccine virus in culture medium containing spinal cord produces a more marked reaction on the skin of rabbits than the virus in medium containing kidney, although the difference is by no means sharp; however, there appears to be almost no difference in the effect of spinal cord and testicle on the virus suspended in the medium.

5. The vaccine virus emulsified with spinal cord causes more marked inflammatory reaction than control glycerinated virus. Therefore, it would appear that the spinal cord of a normal rabbit is favorable to the growth of the virus and tends to increase its virulence.

6. Vaccine virus appears to be incapable of migrating along the spinal cord *in vitro*.

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SPONTANEOUS CENTRAL NERVOUS SYSTEM LESIONS IN THE LABORATORY RABBIT *

LAURETTA BENDER, M.A.

(From the Laboratories of the State Psychopathic Hospital, Iowa City, Iowa)

Recently, various investigators have reported a spontaneous encephalitis in experimental rabbits which has often proved very misleading, especially when the rabbit is used in experiments on the central nervous system. The reports are widely distributed, coming from Twort [1, 2] in England, Levaditi [3] in France, Plaut [4] and Pette [5] in Germany and Doerr [6] in Switzerland. The earliest report was by Bull [7] in the United States. Levaditi,[3] Wright and Craighead [8] and Oliver [9, 10] claim to have found an associated organism which Levaditi calls Encephalitizoön cuniculi. Other reports have been made by Flexner,[11] Wright [8] and Goodpasture and Teague.[13]

These lesions particularly concern us in our laboratories as our work is largely with the central nervous system and several series of experiments had been planned with the rabbit. A careful examination of the brains of all rabbits used during the year of 1924 was therefore made to determine the frequency of the lesions in our stock.

Forty rabbits were used during this year. Twenty-four were used in experiments with insulin, twenty receiving injections to the point of convulsions and death and four being untreated control rabbits. Six rabbits were used in experiments with nerve degeneration and regeneration. Seven rabbits were used in experiments with cholesterol injections. Three young rabbits were born during the course of the experiment and were untreated.

This group of animals included both males and females of all ages from one month to adults weighing nearly 4000 gm. Some had been pregnant and had delivered their young; others were pregnant at the time of death. Clinically some of the rabbits had had snuffles, convulsions and pneumonia. Postmortem evidence of pneumonia, otitis media and liver parasites was found. No relation existed between the experiments performed and the lesions in the central nervous system, although the insulin group showed the least number of lesions of the severe grade. Some relation seemed to exist between

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infections of the upper respiratory tract and the lesions, in that all rabbits showing any marked respiratory symptoms had at least a two plus meningeal reaction, focal lesions or both. However, the rabbit which suffered the most marked clinical symptoms of snuffles had only a three plus meningitis and no focal lesions, while the rabbit showing the most marked histologic picture of severe focal and meningeal lesions had not shown any symptoms.

The meningeal reactions found in the rabbits of our laboratories have been classified as follows: negative reaction in which the brain and meninges do not show definite lesions of this type; one plus in which there is a very slight increase in the lymphocytic cells of the meninges with dilated capillaries throughout the brain, especially of the agranular layer, with slight increase of the cells around these vessels; two plus, the same but more intense; three plus, a marked proliferative meningitis and perivasculitis; and four plus in which there are focal lesions in the brain substance which are always associated with a two and three plus meningitis and perivasculitis.

The number of rabbits having these different conditions were:

Negative	3	rabbits
One plus	20	"
Two plus	7	"
Three plus	1	"
Four plus	9	"
Total	40	"

Associated pathologic findings. The liver usually shows a proliferation of lymphocytes about the triads. The kidneys also have areas of lymphocytes in the interstitial tissue with an occasional small granuloma like the focal lesions in the brain. Numerous eosinophiles are in the spleen.

Description of the Lesions in the Central Nervous System. In the meninges there is an increase in the cells of the subarachnoid space and beneath the pia. These cells are usually small without much cytoplasm. In the more severe cases epithelioid and plasma-like cells with eosinophilic cytoplasm occur. This reaction is most marked over the cerebrum, though it frequently is found about the cerebellum and over the medulla when focal lesions are present there. It is not seen to any marked degree in the spinal cord. The largest accumulation of cells occurs about the points of entrance of vessels into the brain substance.

In addition to the meningeal reaction, the small blood vessels which extend into the cerebrum through the agranular layer are usually dilated and there is a slight increase in the cells in the walls of these vessels. In the mild cases, this cellular exudate consists of cells having small dark nuclei. Half of the rabbits furnished at least this much of a reaction and indeed it was difficult to find a rabbit which could be considered entirely free from some cellular reaction. In the more severe cases, the capillaries throughout the cerebral tissue are dilated and the perivascular proliferation is productive in nature, there being more cells with cytoplasm which is often eosinophilic. The reaction is always most pronounced in the vessels which extend in from the meninges.

The focal lesions are most frequently found deep in the cortex. None is seen in the cerebellum. Several are found in the gray matter of the medulla and midbrain. A few are found in the cord both in the white and gray matter. Careful search will usually show that these are as a rule associated with small vessels which extend in from the meninges. The vessels in question show a perivascular proliferation throughout their course and the lesion appears at the tip like a flower on a stalk.

The lesions are distinctly granulomatous in nature. The center shows a small mass of cell detritus surrounded by cells with irregular nuclei and considerable cytoplasm, the limits of which cannot be readily distinguished. About these are small cells with small dark nuclei and spindle-shaped bodies which often run out into tails. At the periphery of the granuloma in the more severe cases there occur definite spider cells which penetrate some distance into the brain tissue as do the small round cells. Around such a lesion occur numerous smaller ones consisting of round cells lying about the adjacent capillaries.

In a few instances, minute dark staining bodies about two to three microns in diameter have been seen in and about blood vessels, associated with some of the most severe lesions. These may be the bodies described by Levaditi, Wright and Craighead and Oliver as the Encephalitizoön cuniculi. Jahnel's spirochete stain failed to demonstrate any spirochetes.

A phosphotungstic acid hematoxylin preparation shows some, though not many, glia cells which have formed fibrils. Giant, true plasma and polymorphonuclear cells are not seen.

A reaction about the ependyma of the ventricles similar to that associated with the meninges has been described by Oliver and others. But a developmental heaping up of cells in the ependyma of rabbits is frequent and is easily confused with such a condition.

Conclusion

It is quite clear that lesions which are so extensive and occur so frequently render the rabbit unfit for most experiments on the central nervous system.

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EXPERIMENTAL PRODUCTION OF GLIOSIS*

I. EFFECTS ON THE NERVOUS SYSTEM OF THE RABBIT OF INTRAVENOUS AND INTRASPINAL INJECTIONS OF CHOLESTEROL EMULSION

LAURETTA BENDER, M.A.

(From the Laboratories of the State Psychopathic Hospital, Iowa City, Iowa)

A multiplication of glia cells (gliosis) is one of the common reactions of the nervous system to lesions. The reaction is similar to mesodermal scar formation in lesions of other parts of the body. The exact cause of this reaction has not been determined. It may be a purely mechanical replacement of lost tissue; it may be that toxins or other noxious agents which injure or destroy parenchymatous tissue act as a stimulus to the reproduction of the less specialized supportive tissues; or it may be that products of degeneration or the injured nerve tissue act as a specific stimulus to the glia cells. Quite commonly, vascular accidents in the brain destroy fibers of the pyramidal tract in the internal capsule, thus causing a degeneration of these fibers through the midbrain, medulla and cord; and this long tract, far removed from the original lesion, develops a characteristic glia scar throughout its course as a reactive rather than inflammatory process. It was with this idea in mind that a series of experiments were planned in which extracts of brain tissue were to be introduced into the nervous system of animals and the reactions of the glia cells studied.

In this particular experiment, cholesterol was used because it is a lipoid present in the brain in large quantities. Various workers[1] have determined the percentage of cholesterol to be from 1 to 4 per cent in different parts of the fresh human brain. In order to maintain a constant supply of the cholesterol and thus to attempt to produce a chronic condition, the cholesterol was introduced at frequent intervals intravenously and by lumbar puncture.

Merck's purified cholesterol was used in a 1 per cent emulsion. The method of the preparation of the emulsion was somewhat similar to that used by Kaethe Dewey,[2] who recommended pouring an

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acetone solution of the cholesterol into hot water, concentrating as desired and filtering. In our hands, this preparation salted out when sodium chloride was added to obtain a physiologic salt content, or when salt solution, blood serum or whole blood was added. It was found, however, that if an acetone or hot alcoholic solution of cholesterol was poured into a boiling weak saline solution of about one-fourth the strength of a physiologic solution, this emulsion could be concentrated until it was of physiologic strength in its saline content. It was, however, a little stronger than desired in its cholesterol content and could then be diluted with ordinary physiologic saline solution. This emulsion did not break down with time, by boiling or by the addition of salt solution, water or blood serum. Control fluids were made by adding the same amount of alcohol to the weak saline solution and treating this as the emulsion was treated. There was no evidence of any alcohol remaining in any of the preparations.

For intravenous injection, 5 c.c. of a 1 per cent cholesterol emulsion was injected daily into the large vein of a rabbit's ear. One rabbit received twenty injections, a second thirty injections and a third forty injections. A fourth rabbit received twenty-six injections of the control fluid. For the intraspinal injections, cholesterol emulsion of a 1.5 per cent strength was used. Lumbar punctures were made on the rabbits at about weekly intervals, as much spinal fluid being removed as could be obtained and about 1 c.c. of the emulsion introduced at each time. Four rabbits were injected with the emulsion and one with the control fluid.

Careful necropsies were performed and tissue samples taken from brain, spinal cord, liver, kidney and other viscera. Sections after Zenker's fixative stained with eosin-methylene blue were prepared to determine the routine histologic picture. Scharlach R stains of frozen sections of formalin-fixed material gave good results for the detection of cholesterol. Hurst [3] points out that pure cholesterol does not stain with Scharlach R, but stains only if mixed with fatty acids or neutral fats. However, as the bodies here interpreted as cholesterol were found to take the Ciaccio stain as well as to be doubly refractile, it must be assumed that the cholesterol has taken up some fats or that the pure cholesterol is staining in these cells. In addition, Ciaccio's method (Bell[4]) for staining intracellular lipoids was used and found quite as delicate for demonstrating

cholesterol as the usual Scharlach R method. The cholesterol is apparently not lost in any appreciable amounts in the dehydrating agents. It was assumed by Ciaccio that the prolonged chromation rendered the lipoids subject to staining by Scharlach R while the dehydrating process dissolved out the fats and fatty acids. However, there is some question whether chromation does not make fats less soluble to the ordinary fat solvents. The polarizing microscope was used to identify the cholesterol by its doubly refractile character. For the study of the glia cells, Mallory's phosphotungstic acid hematoxylin and Bailey's [5] neutral ethyl violet-orange G method were used.

Protocols of the experiments with the intravenous injections are as follows:

B C. Control. Twenty-six injections of 5 c.c. each of the control fluid were given intravenously over a period of forty-six days. The Ciaccio stain of the liver shows a few cells about the triad containing a definite though slight amount of lipoid substance. In the kidney there is a tendency toward lipoid staining in the interstitial tissues of the pyramids. The Ciaccio stain shows no lipoid substance in the meninges of the spinal cord. In the brain a few round cells occur in the perivascular spaces and a four plus encephalitis of the spontaneous type is present. This is the condition described in the first paper of this series and where it was encountered it was assumed not to be a result of the experiment. By the Ciaccio stain, lipoids are shown in large distinct globules in some of the cells of these areas. The polarizing microscope reveals doubly refractile bodies normally present in the spinal cord and brain and a scattering of doubly refractile bodies in the lobules of the liver. The significant findings in this control animal are: (1) some peribiliary cells containing a slight amount of lipoid which stain by the Ciaccio method, showing that it is not soluble in alcohol or chloroform after the chromating process; (2) lipoids in the interstitial tissues of the kidney; and (3) lipoids in the cells reacting to the spontaneous meningitis.

B I. A male rabbit received twenty doses of 5 c.c. each of a 1 per cent emulsion of cholesterol, totaling 1 gm. of the lipoid in twenty days. A considerable amount of substance staining with Scharlach R is seen about the triads and in the Kupffer cells in the Ciaccio preparations. The kidney contains areas of interstitial lymphocytes and nodular areas like the encephalitic focal lesions. Lipoid in the inter-

stitial tissues is present as in the control. The brain shows a four plus encephalitis. No lipoid is found anywhere in the brain and there is no evidence of an increase in glia cells. The significant findings in this rabbit are: (1) lack of evidence of cholesterol anywhere except in the liver; (2) absence of gall stones, sclerosis of the aorta, endothelial ingestion of cholesterol or any of the other results of hypercholesterolemia frequently reported in the literature; and (3) absence of any glial reaction to the cholesterol.

B III. A female rabbit received thirty 5 c.c. doses of a 1 per cent emulsion of cholesterol, amounting to 1.5 gm. in thirty-two days. It was killed eleven days after the last injection and was found to be pregnant. Considerable amounts of lipoid material are seen in the mesodermal cells of the liver in the Ciaccio preparations. In the kidney, lipoids are present in the interstitial tissues in the pyramids and extending into the medulla and in the convoluted tubules. Considerable fatty changes have occurred in the renal and hepatic cells. The brain is the seat of a four plus encephalitis. A marginal gliosis has developed and a few cells in the perivascular spaces contain a slight amount of lipoid. The erythrocytes tend to take the lipoid stain. The significant findings are: (1) cholesterol in the mesodermal cells of the liver and also in the hepatic and renal cells; (2) a slight amount of lipoid in the mesodermal cells of the brain which may be normal; and (3) no definite evidence of a noteworthy increase in glia cells of the brain and spinal cord.

B II. A female rabbit received forty doses of 5 c.c. each of a 1 per cent emulsion of cholesterol, totaling 2 gm. given over a period of sixty days. She delivered young, developed snuffles and lost considerable weight. In the Ciaccio preparations of the liver are seen lipoids in the Kupffer cells and cells of the triads. There is about twice as much as in B I, which received half as much cholesterol. In the brain is a two plus meningitis and lipoids occur in some of the cells of this reaction. Doubly refractile bodies of the tissues are normal except for an increase in the liver. Significant findings are: (1) evidence of cholesterol deposits in the liver and kidney in amounts directly proportional to the amount given; (2) a possibility that cholesterol has been deposited in the meningeal and perivascular cells of the encephalitic reaction in the brain as a result of the injection.

Final Summary of Results by Intravenous Injection

When cholesterol was injected intravenously it was deposited in increased amounts in the mesodermal cells of the liver in proportion to the amount given, although the rabbit receiving none showed a slight amount. It may be deposited in increased amounts in the pathologic cells (mesodermal) of the meninges and perivascular

TABLE I

Results Obtained by Intravenous Injection of Cholesterol

Rabbit number	Total cholesterol injected	Brain			Liver		Kidney	
		Meningeal lipoids	Perivascular lipoids	Glial reaction	Mesodermal lipoids	Hepatic lipoids	Interstitial lipoids	Renal lipoids
	gm.							
B C	0	+	?	−	+	+	++	+
B I	1	−	−	−	++	+	++	+
B III	1.5	±	+	−	+++	++	+++	+++
B II	2	++	++	−	+++	+	++	+

spaces in spontaneous encephalitis. The control animal showed more than the one receiving 1 gm. but less than those receiving 1.5 gm. and 2 gm.

B III, although not receiving as much as B II, showed more marked deposits of cholesterol. This may have been due to pregnancy or to the fact that the encephalitis was more severe in this animal. The fact that the erythrocytes stained more brightly may be evidence of a greater lipoid accumulation in the tissues of this animal or may indicate that for some reason the lipoids in this animal have stained more readily than usual. Lipoids are normally present in the interstitial tissues of the pyramids of the kidney but they may be increased in the hypercholesterolemia, and while those normally present are not doubly refractile, with the intravenous injection of cholesterol refractile bodies tend to occur. No evidence of any sclerosis of the aorta or of gall stones was found, nor of any increase in cholesterol in any of the tissue elements of the nervous system or of any glia or nerve-cell reaction to the hypercholesterolemia.

Discussion

Several investigators have produced experimental hypercholesterolemia and have reported changes such as sclerosis of the aorta, formation of gall stones and hepatic and renal lesions in animals some of which had received no more cholesterol than the rabbits of this series. In Dewey's experiments, three rabbits which had received 0.2 gm., 2 gm. and 2.4 gm. intravenously, showed all of these lesions in one or more of the animals. Bailey [6] produced sclerosis of the aorta by feeding cholesterol or egg yolk. But it is hard to say how this compares with intravenous injections of pure cholesterol.

Table 2

Results Obtained by Intraspinal Injection of Cholesterol

Rabbit number	Total cholesterol injected	Lumbar punctures	Death after last lumbar puncture	Thickening of meninges	Lipoid in meninges	Gliosis in cord
	gm.					
S C	0.000	2 in 2 weeks	7 days	–	–	–
S I	0.045	3 " 2½ "	9 "	+++	+++	–
S II	0.045	3 " 2½ "	7 "	+++	+++	+
S III	0.060	4 " 5 "	2 "	++	++	+
S IV.....	0.075	5 " 6 "	35 "	–	–	+

Tunnicliff [7] reports that cholesterol in small quantities increases phagocytosis both *in vivo* and *in vitro* but in larger quantities it decreases it. Similar results were obtained by Dewey and Nuzum.[8] This may have been a factor in the increased content of lipoid substance in the cells of the meningeal and perivascular reaction in the encephalitis of our animals. However, this work only definitely demonstrates an ingestion of the cholesterol by the mesodermal cells of the liver and possibly by the reactive cells in the brain which are present as a result of this spontaneous encephalitis. There is also a possibility that the lipoid content of the liver and kidney cells and of the erythrocytes is increased. These very moderate results were obtained in spite of the fact that two of the rabbits were pregnant, which is supposed to increase the lesions produced by the hypercholesterolemia.

As far as known, there has been no previous work on the effects of a hypercholesterolemia on nerve tissue. Parhon and Parhon [9] re-

port an increase in cholesterol in the blood in chronic alcoholism, senile dementia and some paralyses, while there is a decrease in dementia and melancholia. Pighini [10] records finding cholesterol in the spinal fluid in 68 per cent of the cases of paresis, often in epilepsy and in dementia praecox but none in the other conditions. Weston [11] reports that cholesterol can be found in the cerebrospinal fluid in all cases of mental disease, the amount having no relation to the type of the psychosis.

With the hope of bringing the cholesterol into close contact with the nerve tissue, intraspinal injections were made in rabbits. Lumbar punctures were done about once a week and 1 c.c. of a 1.5 per cent emulsion in physiologic saline was introduced after spinal fluid was removed.

Protocols of these experiments are as follows:

S C. A female rabbit received two intraspinal injections of the control fluid with an interval of two weeks between. Death followed one week after the last injection. The meninges of the cord are normal. No unusual lipoids are seen by the Ciaccio stain.

S I. A male rabbit received 3 c.c. of a 1.5 per cent cholesterol emulsion in three intraspinal injections in two and a half weeks and died nine days after the last and twenty-five days after the first injection. It received 0.045 gm. of cholesterol intraspinally.

The dura and arachnoid membranes of the cord are very much thickened, especially over the posterior surfaces. In the dura the thickening is fibrous but in the arachnoid it consists of large vesicular cells. In the Ciaccio preparations these cells are filled with lipoids. The Scharlach R preparations show the same picture very well. Kubie and Schultz [12] claim that the arachnoid cells are non-phagocytic and that phagocytes in the meninges are special cells which multiply rapidly in response to irritation. In any case, in these experiments there is a very striking increase in the large phagocytic cells in the arachnoid space. Bailey's stain for neuroglia fibrils shows a normal glia content in the cord. The polarizing microscope demonstrates doubly refractile lipoids in the cells of the arachnoid space. The significant findings are: (1) a fibrous increase in the dura and a cellular increase in the arachnoid, in which the cholesterol is found; and (2) no glia or nerve-cell reaction.

S II. A male rabbit received 3 c.c. of a 1.5 per cent cholesterol emulsion or 0.045 gm. of cholesterol by three intraspinal injections

over a period of two and one half weeks. Death occurred seven days after the last injection. The spinal cord shows a cellular increase in the arachnoid and axonal chromatolysis of some of the anterior horn cells. The Ciaccio and Scharlach R preparations demonstrate lipoid in considerable quantities in cells of the arachnoid. In an area of trauma are extravasated erythrocytes and an increase in the glia fibrils. A slight increase in the glia cells is present but may be associated with the trauma. The polarizing microscope reveals doubly refractile droplets within the arachnoid cells. The significant finding is an increase in the arachnoid cells which ingested the cholesterol.

S III. A female rabbit received 4 c.c. of the emulsion or 0.06 gm. of cholesterol by four intraspinal injections in six weeks. She was killed one week after the last injection and found to be pregnant. The significant findings are: (1) an increase in cells about the area of trauma and the vessels and in the meninges, with an ingestion of cholesterol; and (2) a moderate glia reaction in the cord.

S IV. A female rabbit received 5 c.c. of a 1.5 per cent cholesterol emulsion or 0.075 gm. in five intraspinal injections during a period of six weeks. She delivered young during this time and was in a poor condition when killed eleven weeks after the first, and five weeks after the last injection. The meninges of the spinal cord are at no place thickened beyond the normal limits nor has any increase in the cellular contents of the arachnoid occurred. The Ciaccio preparations give no evidence of an abnormal lipoid anywhere in the meninges or elements of the cord. Bailey's stain shows a moderate increase in the glia cells of the periphery of the cord adjacent to the large deposits of cholesterol in the meninges. No unusual amount of doubly refractile bodies can be seen with the polarizing microscope. The significant findings are: (1) a moderate gliosis of the spinal cord; and (2) no increase in the dura or arachnoid and no cholesterol deposits at the site of the injection in the cord. On the whole the results were surprisingly negative considering the amount of cholesterol injected. This rabbit received a good deal more than any other and spinal fluid removed a week to ten days after the injection was found on several occasions to develop cholesterol crystals when allowed to evaporate. However, five weeks had elapsed between the last injection and the time of death, during which period the extraneous cholesterol had apparently been removed. The meningeal reaction, which presumably was as great in this animal

as in the others, must also have disappeared leaving only a moderate gliosis.

Conclusions

The conclusions from this series of experiments as far as the nerve tissues are concerned are that a hypercholesterolemia in the relatively small amounts used here has no effect on the cells of the nervous system except possibly to increase the lipoid content of the phagocytic cells of the meninges or perivascular spaces, or in focal lesions already present due to other causes. Introducing the cholesterol directly into the subdural space of the spinal cord causes a thickening of the dura and a marked increase in cells of the arachnoid space which are phagocytic and ingest the cholesterol, and a moderate increase in the glia cells of the periphery of the cord. Five weeks after the last injection the meningeal and arachnoidal reaction is not seen, though the gliosis persists.

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EXPERIMENTAL PRODUCTION OF GLIOSIS *

II. REACTION OF BRAIN TISSUE TO THE LIPOID FRACTIONS AND TO THE RESIDUE OF BRAIN EXTRACTS

LAURETTA BENDER, M.A.

(From the Laboratories of the State Psychopathic Hospital, Iowa City, Iowa)

The striking difference in the chemical structure of brain and spinal cord tissue and the other tissues of the body lies in the high lipin content of the brain and cord. These lipins do not include the neutral fats but consist almost entirely of the more highly complex lipoids, the most important of which are cholesterol, phospholipin and glycolipin or cerebroside. These lipoids are important constituents in all of the cells of the body and their large proportion in the nervous tissue is not due to a cellular increase but to the fact that they are the principal constituents of the medullary sheaths. This is brought out by a comparison of analysis of brain tissue taken from the white matter such as the corpus callosum and the gray matter or child's brain in which medullation is still incomplete.

It would seem possible that one or all of these lipoids might be an important factor in the production of gliosis in the degeneration of the long fiber tracts, such as the pyramidal tract, when no inflammatory process is present to provide stimuli for the multiplication of the glia cells. It is our intention, therefore, to extract brain tissue and obtain these lipins and the residue and to determine the effect of these substances upon normal brain tissue.

Dogs were given ether anesthesia, the femoral vein cannulated, the femoral artery on the same side opened and the blood washed out of the animal with physiologic saline as long as the heart continued to beat. As soon as the cardiac action ceased, the common carotid arteries were cannulated, the jugular veins opened and washing with physiologic saline by gravity was continued until the returns were clear. The brains were removed, stripped of all meninges and superficial vessels and delivered to the chemical laboratory of the hospital, to be extracted by the method given in Mathews' Physiological Chemistry.

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A precipitate is obtained by cooling a hot alcoholic extract. The ether soluble substances are then removed and the remainder consists chiefly of the cerebrosides. To the ether extract thus removed is added the cold alcoholic filtrate; acetone soluble substances are removed and the remainder is chiefly phospholipins. The acetone extract from the above is chiefly cholesterol. The residue from these extractives contains the nucleo- or phosphoproteins. The first three

TABLE 1

Composition of the Brain. Thudichum [1]

(Corrected for dried tissue)

	Corpus callosum	Gray matter
	per cent	*per cent*
Neuroplastin	30.0	53.0
Ether extract (Phospholipin and cholesterol)	38.0	18.5
Cerebrosides and myelin	23.0	2.5
Water extracts	4.7	3.3

White matter exceeds gray matter in phospholipins and cholesterol by 38 to 18.5.
White matter exceeds gray matter in cerebrosides by 23 to 2.5.

Composition of the Brain. Koch [1]

	Corpus callosum	Child's brain
	per cent	*per cent*
Protein	27.0	46.0
Extractives	4.0	12.0
Cerebrosides	18.0	7.0
Phosphatides	31.0	24.0
Cholesterol	17.0	1.8

White matter exceeds child's brain in phosphatides and cholesterol by 48 to 26.
White matter exceeds child's brain in cerebrosides by 18 to 7.

may be considered to represent roughly the materials chiefly derived from the medullary sheaths, and the fourth those from the cells.

These substances were prepared in a sterile condition by evaporating the cerebrin out of methyl alcohol, the phospholipin out of acetone, the residue out of ether and by heating the cholesterol to 120 C. for two hours. These were then preserved in sealed tubes.

Trephine openings were made over the parietal area in dogs under ether anesthesia, the meninges snipped with the scissors, a hole

made in the brain with a small trocar and a piece of the extract pushed through the hole. Five dogs were operated on, one serving as a control while each of the others received one of the extracts. The dogs were allowed to live four weeks. Pieces of the injected area were fixed in formalin, Zenker's solution and Ciaccio's solution. They were stained with Scharlach R, Ciaccio's stain, eosin-methylene blue, iron hematoxylin, phosphotungstic acid hematoxylin, neutral ethyl violet-orange G and the aniline blue connective tissue stain. The material was also studied with the polarizing microscope.

Control. No evidence of infection found. The meninges are moderately thickened at the point of incision. The hole in the brain can be readily seen.

A section through the area of the lesion shows moderate hemorrhage with phagocytes containing blood pigment. The blood vessels in and adjacent to the lesion are prominent. The endothelial cells are slightly increased in the perivascular spaces and the mesodermal fibrous tissue is slightly increased within the area as shown by the aniline blue connective tissue stain. A moderate gliosis of the spider cell type and a few cytoplastic glia cells have appeared adjacent to the lesion.

The significant reactions to this lesion were an increase in endothelial cells and fibroblasts of the blood vessels with some invasion of the area of the lesion, phagocytes full of blood cells and débris, and a moderate gliosis.

Phospholipin. The phospholipins are contained in the ether extract of the brain tissue. They are one of the major constituents of the so-called protagon of the medullary sheaths and also of all cellular protoplasm. They are characterized by Mathews [1] as being a "water-containing, semifluid, highly-reducing, auto-oxidizable, semilipoid crystalline substratum, which contributes to or makes possible the vital phenomena." They are also important in immunity and it is suggested that they are an important factor in staining of the living cell by basic dyes. They constitute 31 per cent of the dried white matter (Koch) and may thus be an important factor in producing gliosis in tract degeneration.

The important phospholipins of the brain are lecithin, cephalin, myelin, paramyelin and sphingomyelin. The chief difference in these substances is apparently in the fatty acid radicle. Lecithin has been the most studied and it is the only one whose formula is

known. On hydrolysis it yields glycerine, oleic acid, stearic acid, phosphoric acid and choline. Thudichum[1] claims that the chief properties of lecithin are due to its oleic acid. For our purposes, however, the fatty acids may prove of less interest than the phosphoric acid and choline.

Choline is a nitrogen base and has powerful physiologic action. By intravenous injections of minute quantities it increases the blood pressure (Mendel et al.[2]) and by intracerebral injection it produces convulsions and paralysis (Donath[3]). Coriat[4] speaks of it as "the most important decomposition product of lecithin which is found in the central nervous system, blood and cerebrospinal fluid in conditions where active degeneration is taking place." He claims that it is found in cerebrospinal fluid, brain and cord only with active medullary sheath degeneration which leads to decomposition of the lecithin and to a coincident increase in phosphoric acid in cerebrospinal fluid. Donath[5] claims that choline and glycero-phosphoric acid pass into the fluid on decomposition of lecithin while stearic acid combines with glycerol to form neutral fats which accumulate beneath the neurolemma.

The extract used in this work must be considered an impure mixture of the various phospholipins and specific results are not to be expected.

The trephine opening is filled with fibrous tissue and the meninges are adherent to it and the underlying brain at the point of the lesion. The meninges are thickened at this point. A vessel in the meninges apparently draining this area is very conspicuous by its size and the thickness of its wall.

On cutting the sections, the microtome knife met with considerable resistance at the point of the lesion. The knife was nicked and the tissue torn. This was apparently due to calcification of the injected phospholipin occurring within the four weeks during which it had been in the brain. Among the various theories of pathologic calcification, the best accepted is the formation of calcium-binding substances within degenerating areas, especially phosphoric acid and fatty acids (Wells[6]). It has been suggested that calcium soaps are formed from the fatty acids and are then transformed into the less soluble phosphates and carbonates. Cellular lecithin and nucleoproteins have been considered as the probable sources of phosphoric acid and fatty degeneration has been recognized as a

precursor of calcification. However, Wells questions these theories. In a chemical study of a large quantity of material he found only traces of calcium soaps in calcifying matter even in the early stages (Wells [7]). He has implanted in the abdominal cavity of rabbits various tissues that had been killed and sterilized by boiling and found that the tissues rich in nucleoproteins show no greater tendency to calcify than those poor in nucleoproteins. However, the residue used by the writer, which presumably consisted largely of nucleoproteins, also produced substances in the brain giving marked resistance to the microtome knife, though it was not nicked. None of the lesions produced by the other extracts seemed unusually hard. This seems to suggest that the phosphoric acid may play some part in calcification. The phospholipins, however, contain both phosphoric acid and the fatty acids.

Studies of the lesion produced by the phospholipin show a picture very similar to that of the control as far as the mesodermal elements are concerned. Hemorrhagic elements are present with phagocytes containing blood pigment. The blood vessels in and about the lesion are prominent because of a slight endothelial and fibroblastic increase. The area contains some fibrous tissue. However, more phagocytes occur here than in the control lesion and many of these contain a substance stained by Scharlach R. Gliosis of the fibril-forming type is more marked but still moderate.

It would appear then that except for the calcification, the hypertrophy of the meningeal vessels and the greater gliosis, the effect of introducing phospholipins into the brain was no greater than the mechanical effect of the trocar hole as seen in the control animal. Possibly if this extract were further broken down into its constituents, namely, pure lecithin or choline, phosphoric acids and the fatty acids, a more pronounced reaction would be obtained.

Cerebrin. The cerebrosides are glycolipins and phosphorus free. They are obtained from the hot alcoholic extract. On hydrolysis they yield a sugar, usually galactose, various fatty acids and a nitrogen-containing alcohol. The common ones found in the brain are phrenosin, kerasin and cerebron. They are not present in the embryonic brain but increase with medullation and constitute 18 per cent of the dried adult corpus callosum (Koch). Apparently less work has been done with the cerebrosides than with the phospholipins from either the chemical or the biologic point of view.

Ossification has occurred in the trephine opening in the animal which had received the cerebrin. The meninges are thickened at the point of the incision. The sections show a microscopic picture somewhat similar to those described above. Hemorrhagic elements are present and the small blood vessels are prominent just as they were in the control and phospholipin specimens. A little more fibrosis has occurred in the area with a slight tendency to capsule formation. There is considerably more gliosis both of the fibril-forming and the cytoplastic type adjacent to the area of the lesion. The amount of phagocytosis is about the same as in the phospholipin experiment.

The gliosis was the most characteristic feature though it was not as marked as that seen in the cholesterol experiment (to be discussed later). It was quite decided and will probably justify further experiment.

Cholesterol. Cholesterol is a sterol or solid alcohol. It constitutes 17 per cent of dried corpus callosum (Koch) but it is also an important constituent of all cell protoplasm. Its empirical formula is $C_{27}H_{45}OH$. It is an unsaturated mono-alcohol and belongs to the general group of terpenes. It is associated with numerous physiologic and pathologic phenomena. Thus it is supposed to prevent hemolysis of the erythrocytes, to check lipolytic enzymes and in this way control the metabolism of fats, to be the mother substance of the bile salts and hence regulate fat absorption and to regulate the water metabolism of cells. It is one of the constituents of gall stones and is found in sclerosis of arteries. It is present in old degenerative processes such as old infarcts, exudates and degenerating tumors in the brain or in other parts of the body. Wells [6] thinks that its action in the cell must be purely a physical one since it is chemically inert. He also speaks of it as behaving like an inert foreign body in pathologic conditions and as being removed by giant cells and foamy endothelial cells. In the previous paper of this series it was shown that an experimental hypercholesterolemia had no effect on the brain tissues of rabbits. When it was introduced subdurally by lumbar puncture, however, there was a fibrous increase in the dura and in the cellular content of the arachnoid, with phagocytosis of the cholesterol and a slight gliosis in the subjacent spinal cord tissue.

In the dog brain there is no gross evidence of infection. The meninges are slightly thickened and adherent as in the other cases. The microscopic picture of the section is striking. Polymorpho-

nuclear cells are sprinkled around a few of the blood vessels and it will therefore be necessary to repeat this work to eliminate the possibility of infection, although these cells might have been called forth by the extract itself. A definite fibrous capsule has formed around the injected substance with ingrowths of fibrous tissue toward the center. The blood vessels around the area are thickened by an increase of their endothelial cells and fibroblasts. Numerous phagocytes are present but these are outside the fibrous capsule which walls off the cholesterol and seems to be associated with the hemorrhage and tissue injury. The gliosis both of the fiber-forming and cytoplastic type is marked, the latter being very conspicuous in places. Except for the gliosis this reaction corresponds closely with that of the spinal cord of the rabbits in which a fibrous increase in the dura and a cellular increase in the arachnoid were produced. The cholesterol was taken up by the phagocytes while in the brain of the dog it was walled off by the fibrous capsule. This difference in the glia reaction may be due to a difference in the substances injected as that used in rabbits was Merck's purified cholesterol while that used in the dog was extracted from a dog's brain.

Residue. The residue is the solid substance left after the brain has been extracted with hot alcohol and ether and may be considered to be the precipitated proteins. It is probably largely nucleo- or phosphoproteins and undoubtedly many other substances. Little is yet known of the nucleic acid of the brain. It yields on hydrolysis phosphoric acid, a purine base and a hexose (Mathews [1]).

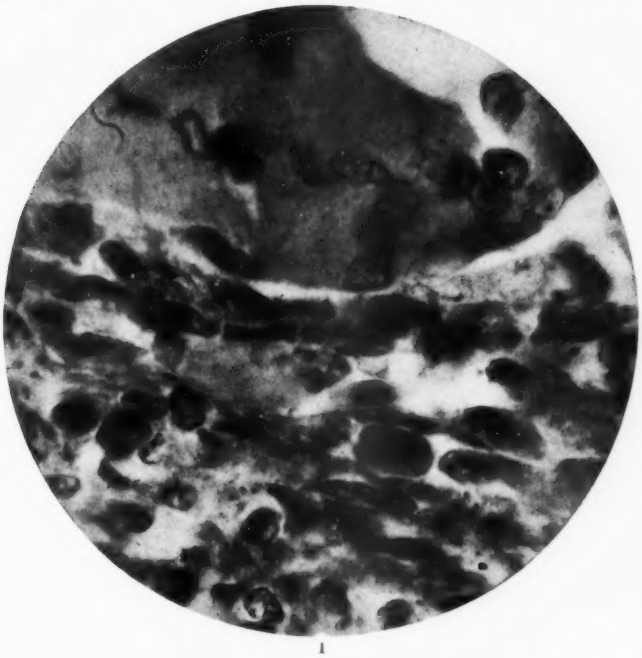
In the animal injected with this residue, the brain in gross looks like the others. Microscopically, a conspicuous fibrous capsule surrounds the injected mass like that in the cholesterol experiment. Outside of this capsule is seen some hemorrhage at one place and also a little tissue destruction. Strikingly few phagocytes occur and the gliosis of the brain tissue is less than in any of the specimens except the control. The striking part of the picture is the heavy fibrous capsule which differs from that in the cholesterol specimen in the number and form of the nuclei. These are numerous and very bizarre in their shape. In addition, a border of syncytial tissue with numerous nuclei and a frothy cytoplasm which seems to be invading the foreign substance with tongue-like projections has developed just inside the capsule. This tissue has been interpreted as a phagocytic syncytium. Such tissue formations are of very considerable

interest. The products of nuclein hydrolysis are said to stimulate cell growth (Wells,[6] and Calkins et al.[8]) and such an intense and bizarre nuclear reaction as seen here will certainly justify further studies with this substance and also further experiment to determine whether the active factor is the nucleic acid or some other ingredient.

Conclusions

One of the interesting results of these experiments is the way each picture overlaps the next. If we may consider each of the four extracts as a sort of fractional product with a predominating substance but containing lesser quantities of the others, we may explain the overlap.

The effect on the blood vessels is about the same in all of the series including the control. These vessels are more prominent in and about the area of the lesion and show an increase in the endothelial cells and the fibroblasts. Calcification was evident in the phospholipin and suggested in the phosphoprotein experiments. The phagocytic reaction varied directly as the amount of hemorrhage and tissue destruction and inversely as the amount of encapsulation. The fibrous reaction within the lesion and capsule formation were least in the phospholipin injection and a little greater in the cerebrin experiment, there being an indication of capsule formation. A definite capsule had developed in the cholesterol experiment with invasion of the mass by fibroblasts, but still numerous extracapsular phagocytes were to be found. In the residue experiment the capsule formation was complete. The rapid nuclear production was at its maximum as a reaction to the residue, though a slight suggestion of it occurred in the cholesterol experiment. On the other hand, the fibrous and invasive reactions were at the maximum with the cholesterol extract where bands of fibrous tissue invaded the mass. Gliosis was the least marked in the control and in the residue experiments. It was increased by cerebrin and was marked with phospholipin and perhaps even more so with cholesterol. The fact that purified cholesterol failed to produce any marked gliosis in the spinal cord of rabbits suggests that some substance common to the phospholipin and cholesterol extracts, which is not present in the purified material, serves as the glia stimulant.



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Bender Experimental Gliosis

These experiments have to some degree separated the various constituents which usually form a single picture in a lesion in the brain. To separate these constituents more completely, it will be necessary to obtain either more nearly pure extracts or to fractionate these more completely. Studies in the various stages in the development of lesions resulting from the present series of extracts might also be of value. The water-soluble extracts were not used in these experiments and these may be important factors in the tissue reactions to lesions in the central nervous system. They include alkaloids, organic and inorganic acids, carbohydrates, amino acids and other unknown substances.

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DESCRIPTION OF PLATE CIV

FIG. 1. Oil immersion photomicrograph of the area of a dog's brain injected with the brain residue or nucleoproteins. At the lower edge of the picture is the brain tissue with the cellular and fibrous tissue which has encapsulated the injected mass and above it the phagocytic syncytial tissue which is invading the mass. The bizarre nuclear forms are well shown.

FIG. 2. Oil immersion photomicrograph of the injected brain residue or nucleoprotein (in the upper right-hand sector) surrounded by the syncytial phagocytic tissue (in the lower left-hand sector) and encapsulated by a fibrous tissue (on the left).

A MULTINUCLEATED LIVER CELL CARCINOMA

HENRY S. ROWEN AND F. B. MALLORY

(From the Pathological Laboratory of the Boston City Hospital)

The following example of liver cell carcinoma is reported on account of the unusual character of many of the cells composing it. No postmortem examination was permitted but the character of the tumor is perfectly evident from its histologic structure. The tissue examined was obtained by surgical operation at the St. Elizabeth's Hospital, Boston, and was brought to this laboratory for diagnosis.

Clinical History. O. O., a male, 57 years old, born in Sweden and a laborer by occupation, came to the hospital Feb. 27, 1925, complaining of pains in the right upper abdominal quadrant.

Family History. Negative.

Past History. Influenza in 1920. "Stomach trouble" since October, 1925. Failing appetite. Clay colored stools one week previous to entrance. Intermittent vomiting past month. No cardiac or respiratory symptoms. Frequent micturition.

Present Illness. Pains in right upper quadrant for past two months, extending the past two weeks to left upper quadrant and into both shoulder blades, accompanied on several occasions with nausea and vomiting.

Physical Examinations. Fairly well developed and nourished. Teeth in poor condition. Heart and lungs practically negative. There is a resistant mass felt in right upper quadrant, extending from the costal margin nearly to the umbilicus. This mass moves on deep inspiration and is somewhat tender on palpation.

Laboratory Examinations. Urinalysis negative. White blood count 8,600. Clotting time normal.

X-ray findings by Dr. L. B. Morrison were as follows:

"Gastro-intestinal meal given shows long tubular type stomach. First part of duodenum does not fill out, and pylorus acts as if pressed upon. At 6 hours head of column has reached ascending colon. There is a large amount of the meal in the jejunum and in the ileum which is dilated. At 24 hours head of column has reached the rectum, most of it is in the transverse colon. Pictures of gall

* Received for publication October 15, 1925.

bladder do not reveal any evidence of stone. There seems to be an increased density in gall bladder region. Liver seems large.

"Conclusions. The lack of filling in the pylorus and first part of duodenum I believe is due to some pressure here from something in the abdominal cavity. The ascending colon ought to be checked up by an enema. The dilatation of terminal ileum is due to adhesions in the lower right quadrant."

Operation March 9, 1925. Exploratory laparotomy under ether anesthesia. High right rectus incision; peritoneum opened whereupon there was a profuse gush of thin bloody fluid. Liver was examined and found to be considerably enlarged and entirely studded with relatively small and large cyst-like masses. When opened these masses were found filled with material of the color and consistency of wet cocoa. Small portion of liver excised. Gall bladder appeared normal. Further surgical interference considered useless. Peritoneum and fascia sutured with catgut and skin approximated with silkworm gut sutures. Sterile dressing applied. Recovery fair.

Three days following operation, wound discharged a large amount of bloody fluid which persisted up to the time of his death, March 21, 1925.

Microscopic Examination

Microscopic examination of sections from the liver shows an infiltrating tumor which is spreading everywhere through the smaller blood vessels and often causes marked distension of them The new growth is composed of cells more or less closely resembling those of the liver but of a distinctly larger size. In places they are arranged in columns with sinusoids and very little stroma between them. Occasionally groups of them contain small and large fat vacuoles so that the picture strongly suggests that seen in a fatty infiltrated liver.

Under high power magnification it is sometimes difficult to distinguish tumor from liver parenchyma. With low magnification, however, the distinction is rendered easy by the larger size of the cells, the tendency to basophilic instead of acidophilic staining of the cytoplasm and the lack of lobular arrangement and bile duct formation.

In places the tumor cells surround bile capillaries of which many are distended with fluid and inspissated bile. The unusual feature

of the tumor, however, is the presence in many places of masses of multinucleated cells of various shapes. These cells are often of huge size and each contains several to dozens of nuclei which as a rule are separate but occasionally more or less fused together. In a few of the large cells are bile capillaries distended with inspissated bile.

Careful search reveals mitotic figures in small numbers. Some of them are single but others are multiple and clearly indicate the origin of the multinucleated cells which are true tumor giant cells.

A certain amount of sclerosis is present in the liver tissue between the masses of cancer cells but this may be due to the action of the tumor. Neither hemosiderin nor hyalin can be found in any of the liver cells.

Some of the tumor nodules are necrotic and undergoing solution. The cysts noted on fresh examination evidently arose in this manner from larger tumor nodules.

Discussion

Primary carcinomas of the liver are relatively rare. The records of this laboratory contain nine in 6506 consecutive postmortem examinations. Seven of them are of the liver cell type. Bile capillaries distended by cylinders of inspissated bile are present in some of them and one shows marked fatty infiltration of the tumor cells but none contains giant cells.

The following points of interest in connection with these seven tumors are worthy of note. Two gave rise to metastases in the regional lymph nodes and one was complicated by cancerous thrombosis of the inferior vena cava with metastases in the lungs and extension to the pleural cavities.

One patient was 32 years of age, the others ranged from 45 to 78. All were males but one. The point of greatest interest is the frequent association of this variety of tumor with cirrhosis. This close interrelation, usually put at over 80 per cent, has long been recognized.

In six of these seven cases of liver cell cancer the cirrhosis is well marked. It is of the pigment type (hemochromatosis) in three and combined with hyalin (the so-called alcoholic type) in the other three. The seventh case is doubtful, not enough liver tissue free from tumor having been preserved to permit a positive statement. There is some sclerosis present but it may be due to the tumor just as in the new case reported in detail.

Cirrhosis of the liver, especially of the chronic progressive types (pigment and alcoholic), furnishes the cell changes favorable for the production of cancer. Continual necrosis of liver cells followed by regeneration results finally in the production of a cell free from control which proliferates indefinitely as a tumor.

The pigment type of cirrhosis, which requires probably the longest time for its production, some ten to twenty-five or more years, seems to be the variety most commonly associated with liver cell cancer. Besides the three marked cases cited here, tissues from two others have been sent to the laboratory and reports of similar examples occur in the literature.

Summary and Conclusions

A report is given of a liver cell carcinoma containing many huge multinucleated tumor cells which have originated from multiple mitoses. In addition reference is made to seven other tumors of the same type, but without multinucleated cells, occurring in 6506 consecutive necropsies. Six of the eight cancers are associated with definite cirrhosis which is of the pigment type in three. In the other three, the cirrhosis is a combination of the pigment and alcoholic types.

Judging from these examples and others in the literature, pigment cirrhosis is the type which is most commonly complicated by liver cell carcinoma.

DESCRIPTION OF PLATES CV-CIX

FIG. 1. Liver on right side, tumor on left. X 200.

FIG. 2. Bile capillaries in tumor distended with fluid and inspissated bile. X 200.

FIG. 3. Two types of growth present, an area with cells containing single nuclei surrounded by multinucleated cells. The picture suggests a chorionepithelioma, but bile cylinders are present in bile capillaries. X 250.

FIG. 4. Multinucleated tumor cells: a multiple mitosis in lower center. X 250.

FIG. 5. Multinucleated tumor cells. X 250.

FIG. 6. A huge multinucleated tumor cell. X 300.

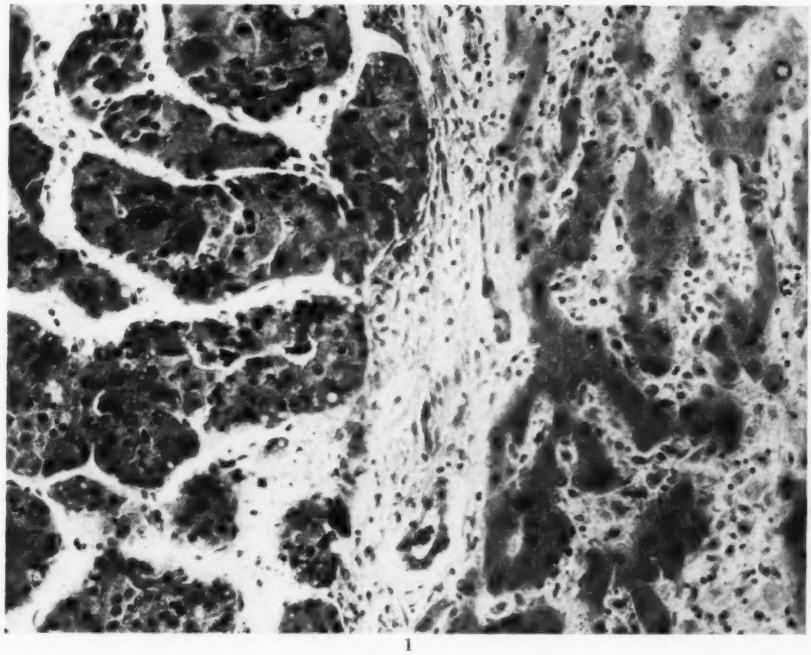
FIG. 7. Cylinders of inspissated bile in bile capillaries of tumor. X 500.

FIG. 8. A multinucleated tumor cell containing two bile cylinders. X 500.

FIG. 9. A large cell containing a nucleus with much chromatin material. X 1000.

FIGS. 10, 11 and 12. Multiple mitoses: FIG. 11 is a detail from Fig. 4. X 1000.

We are indebted to Miss Lillian M. Leavitt for assistance in making the photomicrographs.



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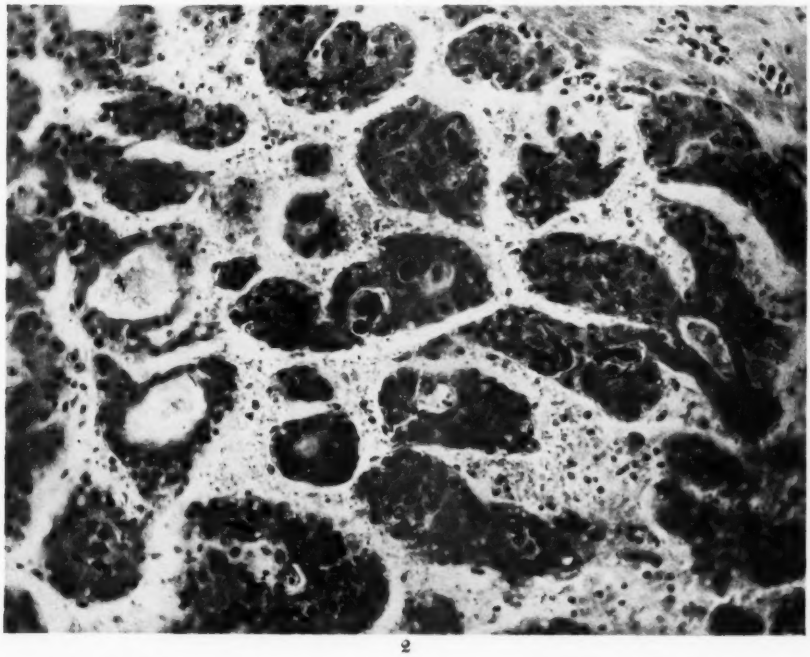

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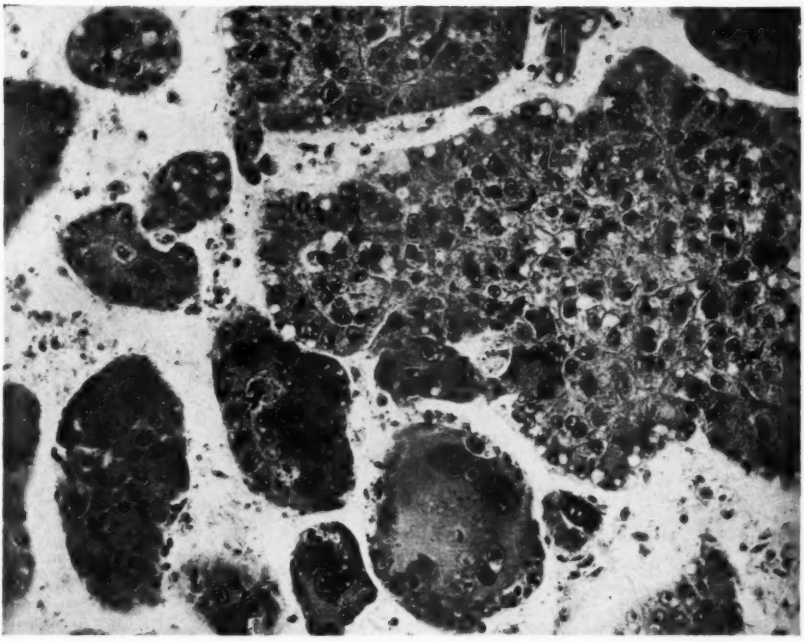
Rowen and Mallory Multinucleated Liver Cell Carcinoma



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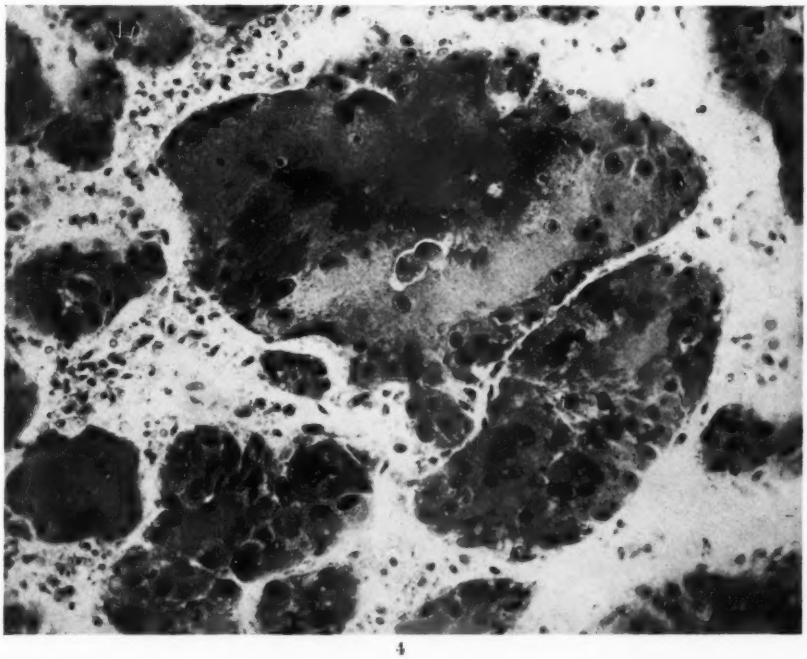

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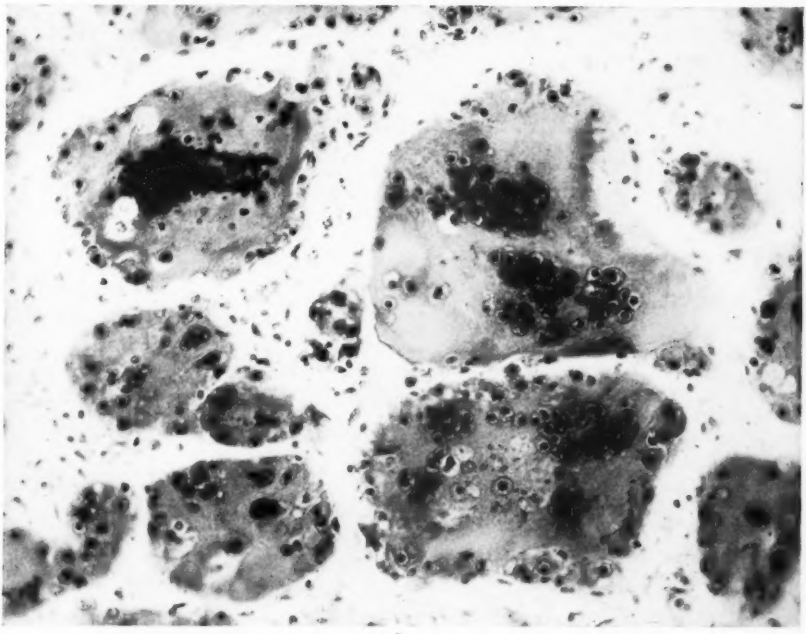

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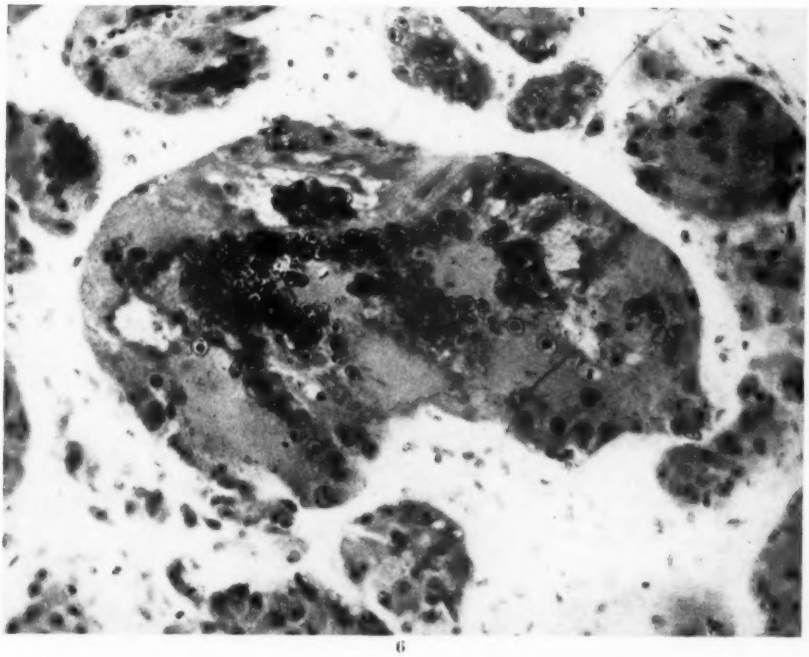

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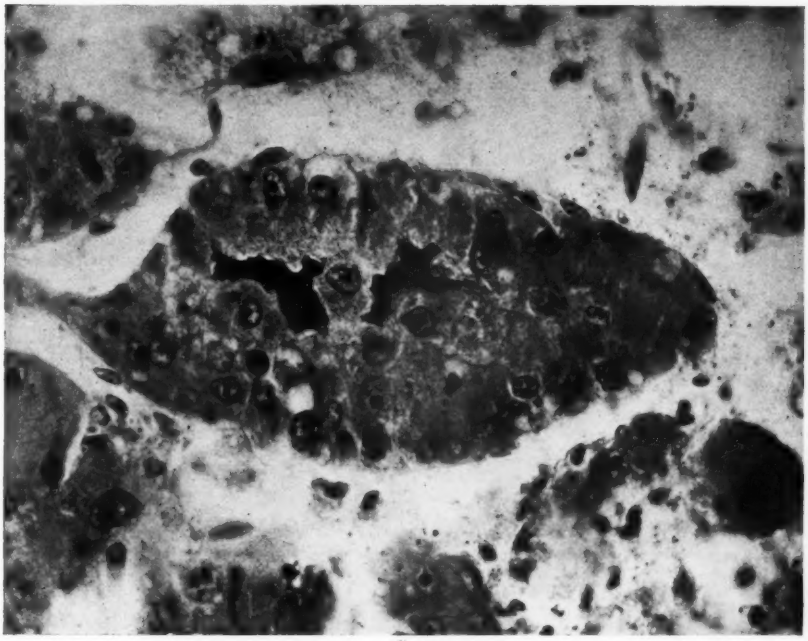
Rowen and Mallory Multinucleated Liver Cell Carcinoma



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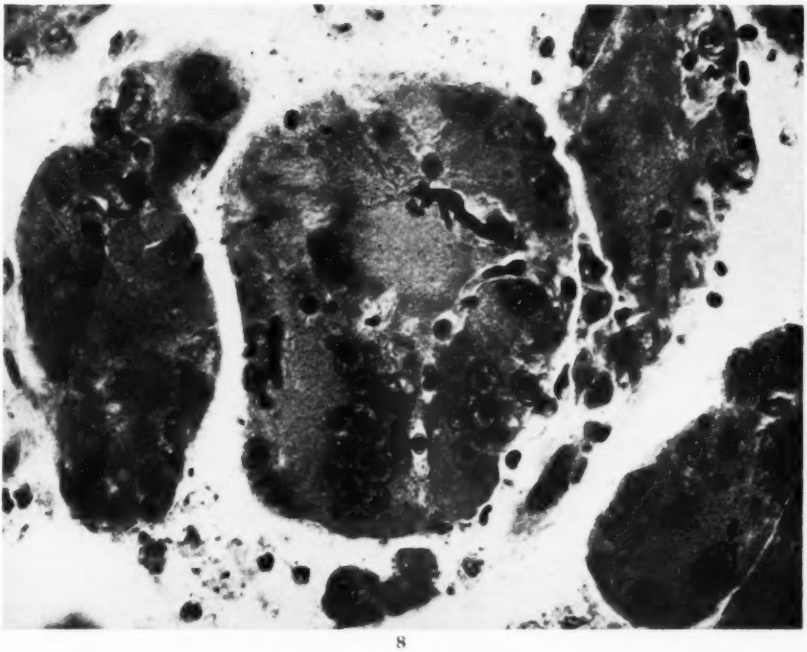

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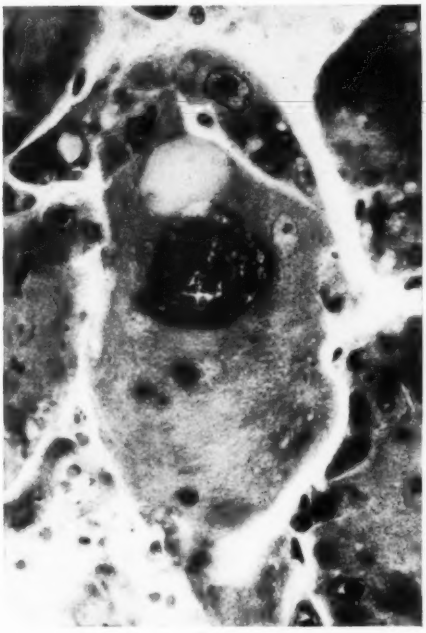
Rowen and Mallory Multinucleated Liver Cell Carcinoma



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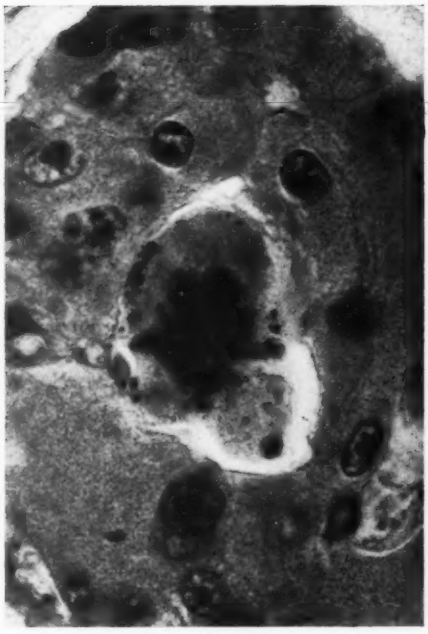

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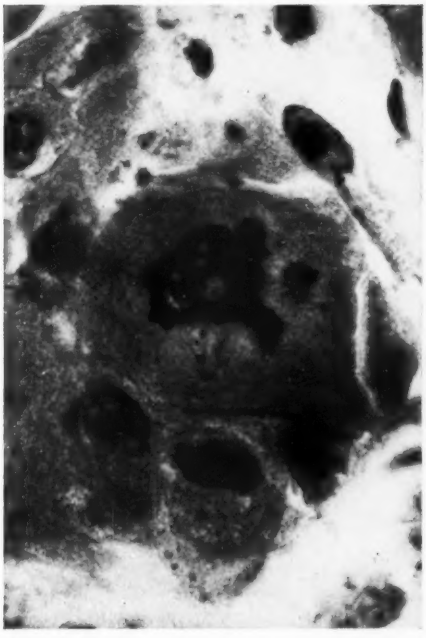

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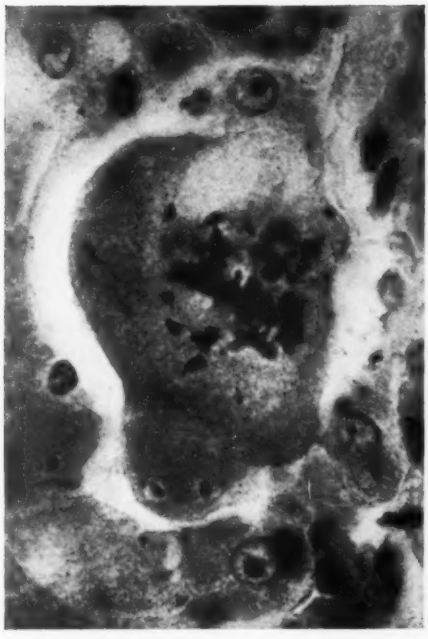
Rowen and Mallory



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Multinucleated Liver Cell Carcinoma

A CORRECTION*

N. C. Foot, M.D.
(Cincinnati, Ohio)

In an article on the occurrence of reticulum in tumors (Foot and Day, 1925), which appeared recently in this Journal, the following statement was made on p. 432: "As nothing appears to have been done in connection with the distribution and occurrence of reticulum in tumors, etc." As I was directly responsible for this statement and as I have received two communications that prove it to be erroneous, I wish to correct it at once.

It appears that White (1900) published an article on this subject from Mall's laboratory, setting forth the results of his investigation of tumor reticulum carried on by means of the Mall and the Spalteholtz digestion methods. His conclusions were very similar to ours, excepting that these methods did not enable him to distinguish clearly between collagen and reticulin.

Furthermore, there is an increasing amount of Italian literature on the subject of reticulum in tumors. Speciale (1924) has reviewed in "Tumori" the work done by some twelve investigators. Of these, one was Japanese and two Spanish, the rest being Italian. Among the articles he quotes, that of Niosi is strikingly similar to our own and our findings corroborate his in almost every particular. He used the Bielschowsky-Maresch impregnation which is essentially the same as the modification employed by us. He finds that reticulum is scarce in benign tumors and has no definite retiform arrangement; it is abundant in malignant growths but is found chiefly in the stroma of the carcinoma, unless that is invaded by wandering tumor cells in which case reticulum appears to be intra-alveolar. He draws attention to the close relationship between reticulum and the supporting tissue of new-growths and to its almost complete absence in the case of hypernephroma. Speciale's review of this article is so excellent that I have not read the original, but have nevertheless listed it at the end of this communication (Niosi, 1914).

Speciale reports his own investigation of a number of tumors in which he studied the reticulum by means of the Achúcarro modifi-

* Received for publication October 12, 1925.

cation of the de Rio-Hortega silver-tannate impregnation. His conclusions are as follows. Reticulum may be found in a great variety of tumors, be they benign or malignant, of connective tissue or epithelial origin; it is, therefore, of little value as a diagnostic landmark in distinguishing between them. It has a definite relationship to the vascular supply of tumors, more particularly to the capillaries and is, therefore, more probably a product of the capillary endothelium than of the tumor cells. He finds that the more mature is the tissue in which tumor reticulum occurs, the less there is of it; while collagen is more abundant in such mature tissue. He therefore stresses the interdependence of reticulin and collagen, believing that the latter is a product of the former, and he quotes Barbacci and Luna as having already emphasized this point.

Achúcarro (1911), in a general article on his silver-tannate method, describes two sets of preparations made from a sarcoma and a carcinoma and compares his results with those of Russakoff who used the Bielschowsky-Maresch method and whose work was quoted in our paper.

I am indebted to Drs. W. C. White and Percival Bailey for calling my attention to these omissions from our article. It is fortunate that our results so closely coincide with those of the authorities just cited. When employing any method of investigation that necessitates the use of a silver impregnation, it would be well to bear in mind that great strides have been made in this direction by the Spanish followers of Ramon y Cajal and by a number of Italian investigators. It is unfortunate that the Spanish and Italian literature is so generally inaccessible in the medical libraries of this country, a fact which largely accounts for our oversight in omitting these references from our article.

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